International Congress on Grapevine and Wine Sciences

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ABSTRACTS BOOK

Instituto de Ciencias de la Vid y del Vino

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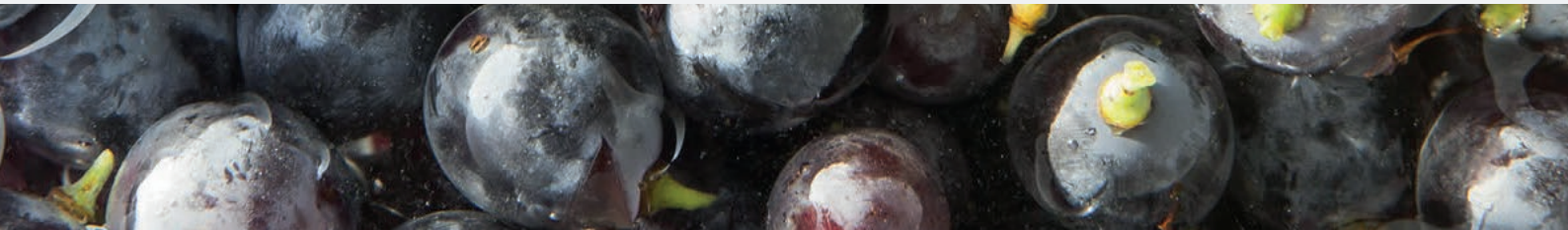

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GN – Grapevine genetics and genetic improvement
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Welcome

Dear colleagues,

The scientific community at ICVV is pleased to welcome you, in the year of the 10th anniversary of the Institute, to the first International Congress on Grapevine and Wine Sciences (ICGWS).

We are very grateful to all colleagues who have been able to clear their agendas and join us during this special event, despite a relatively late announcement of the ICGWS, and the ever-growing offer of scientific meetings, in a particularly dense year. This could be the first meeting of a series aiming to periodically gather together scientist on both grapevine and wine sciences.

For this edition we are hosting around 250 delegates from 18 different countries. We will have the opportunity to discuss with nine key speakers, in a comprehensive program consisting of plenary and poster sessions on almost all topics of interest to ICVV. We invite you to actively participate on discussions around the more than 40 oral presentations and 180 poster communications accepted in the meeting.

We encourage you to join the social program, which includes a visit to our facilities in La Grajera on Friday afternoon, as well as the institutional cellar of La Rioja Government. For some of you this final congress activity might hopefully become the beginning of an enjoyable autumn weekend in La Rioja, which offers natural, cultural, and wine tourism options for all tastes.

Finally, we wish to express our gratitude to our parent institutions, Gobierno de La Rioja, Universidad de La Rioja, and CSIC, as well as all the private sponsors who made this event possible.

We wish you a fruitful meeting and a pleasant stay in La Rioja.

Ramón González

On behalf of the Organizing Committee

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Keynotes

Wine yeasts: from evolutionary history to industrial applications

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Yeasts from the *Saccharomyces* complex have been used for millennia for the production of fermented food and alcoholic beverages. The availability of large genomic datasets during the past decade has improved our understanding of *S. cerevisiae* wine yeast evolutionary history and has provided evidence that these strains have accumulated hallmarks of domestication. We recently showed that evolutionary processes related to anthropogenic niches have shaped the genomes and phenotypes of *S. cerevisiae* and that traits specific to each group can be detected: for example, flor strains are characterized by a higher capacity to metabolize fructose than the other groups and to produce acetic acid, and wine strains by a greater capacity to ferment (Legras et al, 2018). The knowledge of this diversity and the demonstration of the remarkable plasticity of the yeast strain genome may be exploited for further yeast development and pave the way for innovations.

Nowadays, the wine industry faces a series of challenges driven by technological and climate changes, and by customer demand for more diversified products. Continuous innovation includes the design and development of strains with improved robustness, fermentation performance and sensory properties. In recent years, the genetic basis of several technological traits of *S. cerevisiae* has been elucidated through quantitative trait loci (QTL) mapping, and strains improved for various industrial traits have been developed through hybridization or evolutionary engineering.

We will present recent developments based on these approaches. Using serial batch transfer on gluconate -a sugar poorly assimilated by *S. cerevisiae*- as the carbon source, evolved wine yeast strains producing a large amount of esters, which are important determinants of the fruity and floral aroma of wines, have been obtained (Cadiere et al, 2011). Another example is the development of evolved yeast producing wines with more glycerol, less ethanol and increased acidity after prolonged culture under selective salt stress conditions (Tilloy et al, 2014). On the other hand, advances in next generation sequencing have facilitated the identification of allelic variants for industrial traits using QTL approaches. We recently identified in the genome of wine yeast strains 65 QTLs influencing the formation of 30 volatile secondary metabolites. Using reciprocal hemizygosity analysis, we validated the contribution of 13 genes in the observed phenotypic variation (Eder et al, 2018). These allelic variants are involved in nitrogen uptake and metabolism, central carbon metabolism, fatty acid synthesis and regulation, and explain variations in the production of esters, higher alcohols and fatty acids. The detection of these QTLs and their interactions emphasizes the complexity of yeast fermentative aroma formation, and lay the foundation for tailoring *S. cerevisiae* strains with optimized volatile metabolite production for wine.

In the near future, the expansion of large-scale genomic and high-throughput phenotypic data on wine yeast strains will provide a unique resource for elucidating the missing links between genotype and phenotype, further catalysing strain improvement.

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How can grapevine physiology contribute to a sustainable wine production in a changing climate?

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Grapevine physiology made major contributions to improve vineyard management and wine quality over the last decades. Pioneering work, studying carbon uptake and light interception by grapevine canopy was the basis for developing vineyard training systems adapted to local conditions. Concepts of sink-source balance shaped management tools like pruning, irrigation and the appropriate root-stock. Presently, we face an increasingly unpredictable climate, combining seasonal drought with high temperatures, mostly in Mediterranean climates, where a large proportion of vineyards is located. These conditions exert large constraints on yield and quality, posing new challenges on wine production. Paradigms are also changing. Among new practices are the use of genotypes with suitable phenology for the particular climate and stress resistant varieties and root-stocks. Management tools as controlled irrigation, new training systems, canopy interventions and soil management practices can be designed to mitigate excessive solar input and therefore heat stress. Regulated deficit irrigation emerged as a powerful instrument to increase water savings in agriculture, allowing crops to withstand mild water stress with marginal decreases on yield and likely positive impact on fruit quality. Under changing climate, production of high-quality wine will require a continuous monitoring of physiological markers, allowing a timely intervention of the viticulturist. Those markers will be based on advanced knowledge of physiological and molecular mechanisms of plant responses to the environment, particularly available water/temperature. Current research challenges involve studying how the environment regulates genes and proteins of various metabolic pathways responsible for berry development and composition and therefore shapes wine quality. Our success in mitigation/adaptation to a changing climate will rely on multi-strategies combining the different disciplines that come together in viticulture.

Grapevine Breeding: current status and future perspectives

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Biotic and abiotic stresses are the driving forces for breeding resistant and more site adapted grapevine cultivars. Progress in cross breeding can be observed in today´s new grapevine cultivars, which reveal an excellent wine quality. However, the demands on new cultivars is increasing notably due to the challenging climate change and an enhanced demand on sustainability. Thus, additional breeding goals like the adaptation to changes in the ripening regime were introduced during the last decade. Furthermore, long lasting, durable resistance came into reach since genome analyses resulted in numerous genetic loci for resistance against powdery mildew and downy mildew. Some of these loci are in agronomically well adapted genetic background thus permitting to use them for directly breeding of new elite varieties. Other loci are still in a poor genetic background requiring several crosses and steps of selection in order to improve the plants for other relevant agronomic properties e.g. yield and quality traits. Another disease affecting grapevine is Botrytis bunch rot for which no resistances have been identified. Physical barriers resulting in fast drying of grapes like loose bunch architecture and thick, hydrophobic grape berry surfaces are supportive to improve Botrytis resilience. Hence, loose bunches are a major breeding goal in addition to mildew resistances. Further, new phenotyping tools, e.g. based on 2D/3D sensor data, can be applied in order to characterize bunch architecture or other traits of interest objectively and with high-throughput, thus improving breeding efficiency.

The varietal resistance as a sustainable solution for quality wines. The French approach.

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Since the 19th century, vineyards in Europe and especially in France have dealt with the introduction of several grave diseases originating from the United States: powdery mildew (1848), phylloxera (1863), downy mildew (1878) and Black rot (1885). The use of grafting on rootstock resistant to phylloxera and the discovery of the first fungicide treatments helped to limit these crises. Today, chemical control remains the main resources used to limit the impact of these diseases.

Strategies for reducing pesticides

Like for other crops, the possibilities of reducing agrochemicals follow a common approach, but considering the specificity of each sector. This approach defines different levels of pesticide use reduction, from intensive viticulture to organic and biodynamic viticultures (without the use of synthetic products, but allowing the use of other products such as copper and sulfur) and intermediate levels such as reasoned viticulture, with low levels of agrochemicals and integrated viticulture. In the case of intensive, reasoned and integrated viticulture, it should be noted that the effectiveness of many synthetic fungicides has been significantly reduced by the emergence of resistant species. In the case of organic and biodynamic viticultures, excessive use of sulfur and copper in particular exhibits harmful effects on the environment and on soil biology. Therefore, replacing these treatments with varieties tolerant or completely resistant to some diseases will certainly be highly beneficial to viticulture and the environment.

The varietal resistance as a sustainable solution

Varietal resistance already has a long history that begins at the end of XIXth century with the deployment of a first generation of hybrids called “direct producers” (HPD) strongly tolerant to mildew and powdery mildew. A second generation of HPD called “French Hybrids” was established to reach 400 000 ha in 1958, representing 30% of the vineyard at that time (Boursiquot, 1990). Their rapid decline was linked to their mediocre enological and sensory qualities, to the development of effective fungicides allowing the return to the cultivation of varieties of *V. vinifera*. The regulation and creation of AOCs and then of IGPs definitively discarded them as a possible option. Since the Second World War, Germany, Switzerland, Hungary and Italy have continued improving the quality of Franco-American hybrids and some varieties have been included in the wine catalogs of these countries because of their good level of tolerance and quality potential.

A French originality in Europe: the *Muscadinia* strategy

In France, as in the USA, improvement for disease resistance has been resumed in the 1970’s at INRA (Bouquet, 1980, 1986). The particularity of Bouquet’s work was the use of the genus *Muscadinia rotundifolia*, an endemic species cultivated in the Southeast of the United States, totally resistant to powdery mildew and with a very high resistance to downy mildew. From a first hybrid F1, created in the United States at the beginning of the XXth century (Detjen 1916), up to 6 successive backcrossing with different varieties of *V. vinifera* (Grenache, Chasan, Merlot, Cabernet Sauvignon, Fer Servadou, Marselan, Pinot Noir, Ugni-blanc, etc.) allowed to obtain hybrids with very high percentages of *V. vinifera* genome (up to 99%), while maintaining the resistance to powdery and downy mildew from *Muscadinia* (Bouquet, 2003). These new varieties are genetically, morphologically and qualitatively very close to *V. vinifera*.

From these Bouquet’s creations, successive specific programs have led to the selection of several resistant genotypes suitable for the production of very high quality wines (Bouquet, 1980; Salmon et al. 2017), low alcohol wines (Escudier et al., 2017) for the production of Cognac (Ferrari, 2011) or for grape juice (Ojeda et al., 2009). These new varieties, which are characterized by a resistance hypothetically “monogenic” or more precisely “monolocus”, and in any case sustainable in the field which credits the existence of complementary genes inside or outside this locus (Feechan et al., 2015) have already led in a first generation of genotypes to interesting resistant and qualitative genotypes.

They have also had continuity in a second generation of genotypes in which the durability of the resistances will be theoretically secured by introgression of at least two genes of resistance to the same pathogen (Merdinoglu et al., 2009; Schneider et al., 2015). In practice, these varieties called "Resdur", obtained from crosses with hybrids usually of early maturity, have not yet validated their adaptation to the agroclimatic conditions of the south of France in particular and to the Mediterranean regions in general and evaluated regarding their possible adaptations to climate change.

Perspectives of development of resistant varieties in France

These results make it possible to considerer, in France, particularly in the Occitanie region, the first transfers of original varieties improved for both disease tolerance and adaptation to climate warming to the viticulture sector as early as 2019. Within the framework of a National Observatory for the Deployment of Resistant Grape Varieties (OSCAR), these varieties will be temporary registered. This observatory piloted by INRA (UMR SAVE, Bordeaux) aims to organize the monitoring of the evolution of biological aggressors and to identify possible new diseases problems in the absence or a significant reduction of fungicide inputs. The CIVL1 in link with regional chambers of agriculture and IFV2, will be the precursor in Occitanie to establish this innovation at commercial level up to 20 ha for each variety according to French regulation.

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Oxygen management in wine aging. State of the art and the technology involved

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That oxygen is one of the ingredients for successfully improving wines during the maturation process has been a well-known fact since the studies of Ribereau Gayon in 1931. Since then, some work has been carried out to determine how and how much oxygen contributes to the barrel aging processes from the first values determined by Ribereau Gayon to the latest measurements made in the characterization of the OTR of barrels with opto-luminescent technologies. These measurements, carried out in barrels over a year, have shown that oxygen permeation is a dynamic rate that decreases with the length of time the wine remains in the barrel. The key factor determining this change in oxygen permeation by wood is the progressive wetting of the staves.

The limited life of oak barrels led to the emergence of different techniques which attempt to reproduce the supply of oxygen to the wines (in very low doses and continuously, MOX) that is produced in the oak barrel, but at a low cost and with the possibility of modifying the addition of wood. At the end of the nineties, the first equipment appeared that injected oxygen into the wine (active micro-oxygenators) which, together with the contribution of pieces of oak wood, tried to reproduce the barrel, thus imitating the improvement processes produced during the wine's stay in the natural micro-oxygenator of reference. Subsequently, technologies based on the use of materials with oxygen permeabilities like that of wood appeared, theoretically allowing MOX to be carried out in a manner similar to that of the barrel, both in the dose transferred to the wine and the way in which the oxygen is dosed, forming the so-called passive micro-oxygenators. With the use of these alternatives to the barrel and their analysis, it became clear that, with advanced management of the micro-oxygenation dose and/or consideration of the oxygen trapped in the wood porosity, dynamic oxygenation rates were obtained in wines treated with alternative MOX+wood systems. This made it possible to propose the use of both active and passive micro-oxygenation systems, much closer to the natural aging produced in barrels.

Control of the oxygen doses in the alternatives to the barrel, both with active and passive MOX, shows the advantages of having known and homogeneous oxygenation systems between containers as opposed to the heterogeneity in the oxygenation rate of barrels built in a semi-artisanal way with natural materials. Studies carried out on numerous barrels have shown that not all barrels in the same batch behave in a similar way. Work is currently underway to build barrels with a more homogeneous oxygenation rate and with different levels of controlled OTR. The main problem lies in the classification of wood for its capacity to permeate oxygen. The criterion used by cooperages for the classification of wood is by the so-called wood grain: an anatomical characteristic defined by the annual growth ring, a consequence of the growth speed of the trees, which conditions the contribution of wood compounds to the wine they will age. Presumably this classification also affects the permeation of oxygen via the wood, and it has been so used by cooperages. Work is currently being done on the possibility of classifying oak wood for its OTR potential for the construction of barrels and thus enabling cooperages to build barrels with different levels of wine oxygenation. The work carried out by our group showed that, if raw oak staves were classified by their OTR, barrels could be built with different levels of wood OTR. With this background, several initiatives have been started to acquire the knowledge of which factors in the wood determine its oxygen permeability. The anatomy of wood and its handling in cooperages seem to be the factors that jointly affect the amount of oxygen reaching the wine through the wood of the barrels. With this premise it has been determined that there is no single anatomical characteristic capable of explaining the differences in oxygen permeability of French oak wood, but rather that it is the set of its anatomical and physical characteristics that determine the flow of oxygen. To reach this conclusion, the anatomy of wood has been studied in a non-destructive way with helical Nano-tomography of X rays. This, together with the density of the wood, has allowed the future OTR of the wood to be consistently predicted and to be able to classify it by using artificial neural networks. Therefore, it is the set of the main anatomical characteristics of wood that determines its permeability to oxygen, even after the cooperage processes, i.e. the classification of raw wood, allows this class to be maintained in the barrels

built with the classified material. Work is currently being done on the classification of staves for their potential OTR by means of image analysis of photographs of the heads of the raw staves for implementation in the barrel processing line.

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Glycosidic aroma precursors: importance for wine and sparkling wine

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After the first identification of glycosidic aroma progenitors in grapes and wine by Williams *et al.* (*1*) and Günata *et al*. (*2*) considerable progress has been made with regard to their analysis (*3*), structure and reactivity (*4*). One topic that had attracted less attention so far, is the influence of fermentation on the cleavage of the glycosidically bound aroma precursors. In a recent project the use of different yeast strains with regard to selective liberation of aroma compounds from their non-volatile precursors has been studied (*5*).

It could be shown that fermentation considerably influences the sensorial impression of the final product (increase in fruity notes and a decrease of unripe green odor impressions). This indicated the substantial role of odorless aroma precursors in the base wine for the aroma of the resulting sparkling wine. Sixteen wine and nine sparkling wine yeasts were screened with regard to their individual cleavage activities of glycosidically-bound aroma compounds. This screening allowed us to classify them into groups of weak and strong releasing yeasts. With the knowledge of the cleavage potential of the yeasts, the wineries are now able to control the aroma of the wines and sparkling wines. Thus, there is the possibility to recommend e.g. a weak releasing yeast for the production of base wines to yield an aromatic sparkling wine with strong varietal flavor. Consequently, the major part of the flavor compounds in the base wine are present in their more resistant bound form. During the production of the sparkling wine, the bound flavor compounds are released to develop the typical varietal flavor.

In a further part of the study, the focus was put on the release and formation of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), since this compound is responsible for the “kerosene” off-flavor in Riesling wines. It was shown for the first time that yeasts have an impact on the concentration of TDN in the resulting product. This finding together with other measures in the vineyard and cellar will ensure the production of Riesling wines even under rapidly changing climatic conditions.

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Key drivers of wine appreciation and consumption: A cognitive perspective

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What is driving wine appreciation? It has become clear in recent years that cognitive processes play a major role in wine perception and preferences: Experiencing wine is not just a physical experience but also a cognitive and an emotional one. Although the 'intrinsic' quality of a wine (i.e. how the wine tastes) is an important driver of wine appreciation, extrinsic information such as price, name or origin of the wine might modify the way the wine is perceived. For example, Wansink and his collaborators (2007) showed that knowing the origin of a wine (California vs North Dakota) influenced non only the wine rating but also the amount of food consumed [1]. Likewise, Siegrist et al [2] showed that ratings from wine critics influence consumers' actual sensory experience of the wine and Danner et al [3] found that elaborate wine descriptions positively influenced people's emotions, expectations and willingness to drink. In this talk I will discuss some cognitive mecanisms involved in those effects and present some recent work to illustrate the contributions of cognitive science to the understanding of the key drivers of wine appreciation.

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Oral Communications

Aspergillus species associated with wine grapes in Australia: Implications for fumonisins in wine

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Fungi belonging to *Aspergillus* section *Nigri* frequently occur on grapes. Some species within this section produce carcinogenic toxins including ochratoxin A and fumonisins (FB2, FB4) in grapes and grape-derived products. The ability of Australian *Aspergillus* species associated with wine grapes to produce fumonisins has not been investigated to date. Furthermore, little is known about the fate of fumonisins during the fermentation process and their stability in wine.

Aspergillus section *Nigri* strains were isolated from Australian wine grapes to examine their potential to produce fumonisins. The fate of fumonisins during fermentation was studied using Chardonnay grapes inoculated with a fumonisin-producing *Aspergillus* isolate. The stability of fumonisins (FB2) in wine stored at 16°C was studied using white wine spiked with FB2 standard. Liquid chromatography-tandem mass spectrometry was used to analyse fumonisins in all the experimental samples.

Of the 44 isolates collected from seven vineyards in NSW, Australia, four isolates were identified as producers of fumonisins. These isolates produced from 0.2 to 25 mg/kg and 0.7 to 16 mg/kg of FB2 in Czapek Yeast Extract Agar (CYA) and synthetic grape juice medium (SGM), respectively. Fermentation removed nearly 80% of the fumonisins originally present in juice derived from *Aspergillus* inoculated grapes. Fumonisins were further removed during solid separation after fermentation, however; approximately 10% of the fumonisins originally found in juice was detected in final wine. FB2 was shown to be stable for at least three months in the stored white wine. Data from this study demonstrate that fumonisins produced in grapes prior to harvest could potentially be passed on to wine and persist for several months.

Use of BCAs for effective control of *Botrytis cinerea* in vineyards

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Botrytis bunch rot (BBR) – caused by *Botrytis cinerea* – is usually managed by a routine application of fungicides at: A, flowering; B, pre-bunch closure; C, veraison and D, pre-harvest. A 4-year experiment carried out in Italy showed that *Trichoderma atroviride*, *Aureobasidium pullulans* and *Bacillus subtilis*, applied at B, C and D, respectively, provide efficient control of BBR (Pertot et al., 2017). Recently, a new epidemiological model for *B. cinerea* was developed and validated (González-Domínguez et al., 2015). The validation showed that infections occurring during flowering play a key role in determining the severity of BBR on mature bunches. New experiments were performed to verify whether BCA applications, based on the disease model, could provide satisfactory disease control when applied in A too. This study shows that the application of BCAs in A significantly reduced the colonization rate of *B. cinerea* and the sporulation potential in bunch trash, as well as the incidence of latent infections of berries, similarly to a reference chemical fungicide and a botanical. Therefore, the use of BCAs for controlling BBR can be extended to early-season treatments based on the advice provided by the mathematical model, which account for weather data, crop phenology and different infection pathways.

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Protection against fungal grapevine trunk pathogens by *Trichoderma atroviride* SC1 treatments from nursery to the vineyard: a three–year study.

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Trichoderma atroviride SC1, applied at the hydration, callusing and pre-planting stages, has been reported to effectively control infections caused by the grapevine trunk pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* in nurseries. In this research, an experiment was carried out using cuttings of 110 R rootstock subsequently grafted with Garnacha Tintorera cultivar. These materials were subjected to two different management strategies: i) In the 2015 growing season, treatments with a suspension of formulated *T. atroviride* SC1, which were applied during the nursery propagation process. Then, in 2016, they were treated with *T. atroviride* SC1, which was applied at the planting moment in two different commercial vineyards and, later, in 2017 a treatment with *T. atroviride* SC1 at the pruning moment also in both vineyards; ii) Treatments with water (control) at each of the previously described moments in 2015, 2016 and 2017. The effect of the two strategies was compared by fungal isolation from treated and untreated plants on culture media (2015, 2016 and 2017) and by measuring undried shoot weight in the vineyard (2016 and 2017). Results showed that in the three years, grafted grapevine plants treated with *T. atroviride* SC1 in the nursery and in the vineyard showed a higher phytosanitary quality than untreated plants, due to reduced isolation of grapevine fungal trunk pathogens. Moreover, treated plants showed significant higher undried shoot weight after the first growing season in both vineyards.

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Fungal community structure form soil to grapevine root and its interaction with black–foot disease

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The plant compartments of grapevine provide unique niches that drive specific microbiome associations. The majority of phyllosphere endophytes originate from the soil and migrate up to the aerial compartments through the root endosphere. In this study, we investigated how fungal communities differ in different habitats inside and outside of grapevine roots. Grapevine samples were collected at 5 young vineyards in La Rioja which were between 2.2 and 14.9 km distant to each other. Characteristics of these vineyards were: 110 Richter/Tempranillo combination and similar soil, climatic conditions and viticulture management. Three different sample compartments were studied: bulk and rhizosphere soil, and roots. Four samples were collected in June 2017 from each compartment and vineyard. The composition of fungal communities was examined using high-throughput amplicon sequencing of ITS region. Droplet Digital PCR (ddCPR) technology was used to measure the abundance and dynamic changes of fungal pathogens associated with black-foot disease. The fungal diversity was similar in both bulk and rhizosphere compartments at all taxonomic levels. A significant reduction in species richness was found in roots relative to bulk and rhizosphere soil, with distinct community structure especially in lower-order taxonomic analysis. Black-foot pathogen relative abundance decreased from grapevine roots towards bulk and rhizosphere soil, which was significantly correlated with ddPCR results. Results obtained provide helpful information to better understand how grapevine shapes its microbiome and the implications for vineyard management and productivity.

Effect of abiotic stresses on the physiology of grapevine infected by Botryosphaeriaceae

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Botryosphaeria dieback is one of the most widespread and prevalent grapevine trunk diseases (GTDs) in Europe, especially in France. External symptoms, whose incidence and intensity vary from one year to another, consists mainly in bud necrosis, leaf discoloration, shoot dieaback and dead arms. In wood, brown stripe and necrotic sectors, wedge shaped necrosis are present. Causal agents of this disease are Botryosphaeriaceae and among them, *Neofusicoccum parvum* and *Diplodia seriata* are frequently isolated. Their aggressiveness tends to be affected by environmental factors especially temperature that may act as a triggering factor. In this context, our study focuses on the characterization of the influence of two environmental factors, temperature and water stress, on the interaction grapevine / *D. seriata*, *N. parvum*. Cuttings of Ugni blanc and Chardonnay, more or less susceptible to GTDs respectively, were infected by *D. seriata* or *N. parvum*, under two soil water status, with a heat stress (three days at 35°C) or not. Growth plant parameters, the size of necrosis induced by the pathogens and photosynthetic parameters were measured. In addition, transcriptomic and metabolomic analyses are conducted to better characterize the whole plant responses.

Preliminary results showed alterations of photosynthesis under the abiotic stresses tested. *N. parvum* induced larger necrosis than *D. seriata* for both cultivars. For Chardonnay, its aggressiveness seems also to be increased under a heat stress, while it tends to decrease under a water stress combined or not with a heat stress. In addition, modifications in the expression of genes involved in stress tolerance, redox status and defense pathways have been noticed. Similar experimentations are in progress to confirm these observations.

This research included in the GTDfree project was funded by Hennessy & Jas and ANR Chaire Industrielle. It was led in collaboration with Comité Champagne (CIVC) and Bureau National Interprofessionnel du Cognac (BNIC).

Management of grapevine trunk diseases in nurseries: A South African perspective

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Grapevine trunk diseases (GTD) is a serious problem in all countries where grapevines are grown. The causal organisms infect grapevines as early as the propagation process. Managing these pathogens is extremely difficult due to the fact that several taxonomically unrelated fungal species are involved, many if which belong to large fungal complexes. They infect mother vines and propagation material at various stages in the propagation process. Understanding the epidemiology of the pathogens involved is crucial, i.e. to identify inoculum sources and to understand how and when these pathogens are released to infect vines. Rootstock susceptibility towards GTD pathogens and the duration of pruning wound susceptibility in rootstock mother vines are also key aspects. Rootstock mother vine pruning wound protection has been identified as a critical step in efforts to improve the phytosanitary status of rootstock propagation material. Several chemicals and biological control agents are currently under evaluation for their ability to protect pruning wounds. In future, biological control may play an important role in grapevine nurseries. The beneficial properties of *Trichoderma*, with regard to grapevine growth promotion (i.e. increase in root mass) and suppression of various GTD pathogens, has been documented. However, inconsistent results during field applications have been reported. Research is underway to investigate application methods in grapevine nurseries and to study Trichoderma colonisation of nursery vines. Hot water treatment of propagation material and dormant grafted nursery vines is also an important strategy to manage GTD. The effect of a newly introduced HWT-regime in South Africa, namely 50°C for 45 mins, on GTD pathogens is in progress. Ultimately, successful management of GTD's in nurseries will rely on in-depth knowledge of the causal organisms, nursery processes and the integration of multiple strategies to formulate effective integrated management strategies. Due to the complexities of the variables involved, these strategies will most likely differ from country to country.

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Biogenic amines and ethyl carbamate precursors production by indigenous lactic acid bacteria from Rioja Alavesa red wines

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The employment of indigenous strains for wine fermentation has become a common practice. Microorganisms providing sensory desirable compounds but lacking the ability to promote metabolites of safety concern to humans as biogenic amines (BAs) and ethyl carbamate (EC), are essential to develop high quality wines

The aim of the study was to identify safety concern metabolite non-producing indigenous lactic acid bacteria (LAB) of Rioja Alavesa spanish region. To that end 33 samples of must and wine at all stages of vinification were analyzed. 16S rDNA gene sequencing and RAPD-PCR typification showed the predominance of *Oenococcus oeni* during malolactic fermentation (MLF) (>98%). Other LAB species isolated were *Lactobacillus hilgardii*, *Pediococcus parvulus*, *Lactobacillus mali* and *Lactobacillus plantarum*; however, with minor frequency. Genus rarely found in oenological environment such as *Staphylococcus* and *Paenibacillus* were also detected. Although species variety was limited by *O.oeni* abundance, this species showed great genotypic diversity throughout MLF (ID>0,80).

The ability of all isolates to produce BAs and degrade arginine was phenotypically assayed in decarboxylase medium and confirmed by RP-HPLC. AB production potential was restricted in the LAB isolated strains (*O. oeni*, *P. parvulus* and *L. mali*), as they only produced putrescine. However, species of *Staphylococcus* and *Paenibacillus* showed high aminobiogenic capacity; they all produced histamine, tyramine, putrescine and cadaverine. Moreover, a great percentage of *O. oeni* (40%) and *L. hilgardii* (70%) able to degrade arginine was detected, highlighting the potential risk for the formation of EC precursors.

Our results confirm the major adaptation of *O. oeni* to wine fermentation conditions and the low contribution of LAB to AB production. Additionally, they highlight the presence of potential wine spoilage microorganisms in spontaneous fermentations as well as the risk for the formation of EC precursors derived from arginine degradation. The employment of selected malolactic starters could solve those adverse situations.

The influence of yeast on chemical composition and sensory properties of rose wines

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Resumen

Rose wines are gaining importance in the last few years. In fact in the last 15 years the intake of rose wines has increased around 20%. Spain is the second most important country in the world regarding rose wine production (around 5.5 10^6 Hl).

No-*Saccharomyces* yeasts may contribute to achieve both aromatic complexity and quality in wines. Generally, these yeasts are not able to finish alcoholic fermentation, so they are usually used as starters, and subsequently *Saccharomyces* yeast is co-inoculated.

In the current work rose wines were done from Garnacha and Cabernet sauvignon varieties. Three yeast inoculations were tested: i) *Saccharomyces cerevisiae* ii) *Torulaspora delbrueckii* + *Saccharomyces cerevisiae* iii) *Metschnikowia pulcherrima* + *Sacharomyces cerevisiae.*

The microbiology analysis allowed us to determine the percentage of implantation of each yeast in the different treatment in the middle and also at the end of the alcoholic fermentation.

Wines carried out with no-*Saccharomyces* showed lower alcohol degree, color intensity, a* and b* (CIELab parameters); meanwhile higher isobutanol and isoamilc alcohols.

Differences in the volatile profile among both varieties and treatments were found. The use of a chemometric approach allowed us to study the way the wine composition was modulated by these two factors. Additionally, the main volatile compounds involved in this modulation were highlighted for each one. Relationships between yeast and volatile composition of wines were investigated.

At tasting, the higest scores were got by *Saccharomyces cerevisiae* in the nose, and by *Saccharomyces cerevisiae* + *Metschnikowia pulcherrima* in the mouth.

Potentialities of wine yeasts to produce unusual aromas during fermentation

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A current challenge for the beverage sector, due to the highly competitive worldwide market, is to diversify the range of products and to offer wines and spirits with typicity and character.

Wine aroma is a complex mixture of one thousand molecules, with four origins : varietal, pre-fermentative, fermentative and ageing aromas. Secreting hydrolytic enzymes, yeasts contribute to the release of varietal aromas, like thiols. In addition, they are responsible for the synthesis of fermentation aromas. The metabolic pathways involved in the formation of higher alcohols, acetate and ethyl esters, which are the most abundant volatile compounds synthetized by yeasts, have been well characterized. Furthermore, other molecules with a major organoleptic impact can be produced during fermentation. These compounds include terpene derivatives, related to sterol metabolism, benzene derivatives, originating from phenylalanine degradation and lactones coming from β-oxydation of fatty acids. However, little information is available on the yeasts ability to produce these unconventional molecules.

This present work aims to provide an overview of the capacities of the yeast species from the enological consortium to produce these unusual aromas. In this study, fermentations were carried out using 41 yeast strains grown on three different musts (Maccabeu, Ugni Blanc, Chardonnay) and a synthetic must, used as control. The profiles of unusual aroma at the end of fermentation were determined by a dedicated Direct Liquid Liquid MicroExtraction (DLLME) GC/MS method, recently developed in the lab. Interestingly, the yeasts are actually able to produce most of the quantified molecules. However, different production profiles were observed depending on the strains, the species. Moreover the cultivars have also a significant impact on the biosynthesis of these aromas. Overall, this study provide new prospects to extend the aromatic palette of wines and to best meet the consumer expectations for wine quality.

Fostering the utilization of the Non-*Saccharomyces* yeast *Saccharomycodes ludwigii* UTAD17 as a co-adjuvant of fermented beverages through the exploration of comparative genomics data

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Fermentation of alcoholic beverages is traditionally conducted by *Saccharomyces cerevisiae*, however, the essential contribution of Non-*Saccharomyces* yeasts (NSYs) in determining the properties of the obtained product is being increasingly acknowledged. NSYs once considered contaminants are now being re-appreciated for their potential in flavouring wine and beer[1] as they are shown to modulate the aroma profile and/or the alcoholic content of the end product. In this work we have focused on the species *Saccharomycodes ludwigii*, frequently referenced as a spoilage agent, but recently highlighted due to its bio-flavoring potential[2]. To better understand the biology and physiology of *S'codes,* the genomic sequence of strain UTAD17, recovered from wine must, was disclosed using Illumina MiSeq. Data assembly and subsequent manually curated annotation, enabled the identification of 4,025 proteins composing the *S'codes* ORFeome including several proteins predicted to play a role in aroma formation. PFGE-profiling resulted in 7 chromosomal bands and an estimated size of 13 Mbp, consistent with the assembled genome size (~11Mbp). The analysis of the genomics data will be discussed in this work contributing to understand the biology, physiology and ecology of this species and also fostering its utilization as a co-adjuvant in beverages production.

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Identifying the main drivers in microbial diversity for Cabernet-Sauvignon cultivar, from Europe to South Africa.

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Wine consumers have a great acceptance for wines with autochthonous character. This autochthonous character has been linked to different parameters, from weather conditions to soil chemistry but also to grape microorganisms. In the case of this microbial *terroir*, the question of which are the main drivers defining it remains open. This work aims to assess yeast biodiversity and how it is associated with geography and cultivar. The mycobiome of grapes from different vineyards in four wine regions of Europe (Georgia, Italy and Spain) and South Africa was analyzed in local autochthonous wine cultivars compared to Cabernet-Sauvignon cultivars in the same regions. The work design allows us to differentiate three layers of microbial diversity drivers: wine region (Rioja, Tuscany, Kakheti, Stellenbosch), wineries, or local *terroir* (different cultivars sharing growth and climate conditions), and cultivar specific associated microbiota (Cabernet Sauvignon, Tempranillo, Sangiovese, Rkatsiteli, and Chenin Blanch). The use of a metagenomic approach allows us to provide a new perspective on the mycobiome of grapes. Preliminary results show that the country of origin was the main driver clustering the different samples, clearly separating South Africa form the European countries. Also, samples belonging to the Cabernet-Sauvignon cultivars were more similar to each other than samples belonging to different local cultivars, thus suggesting a varietal microbial signature.

Impact of the main characteristics of the must on the outcomes of wine fermentation with non-*Saccharomyces* yeasts in sequential inoculation

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Alcoholic fermentation, an essential step of winemaking, has been mainly controlled over the past 50 years through the use of selected starter cultures of *S. cerevisiae*. This is the safest way to ensure the completion of fermentation and to avoid undesirable off-compounds. The non-*Saccharomyces* yeasts, predominant in grape juice, are rapidly outcompeted by *S. cerevisiae* during fermentation because of their poor adaptation to increasing concentrations of ethanol and the lack of oxygen. Even though these species were used to be considered as spoilage microorganisms, their potential to improve the sensory quality of wines is now recognized. Although the behaviour of some non-*Saccharomyces* yeasts and *S. cerevisiae* were studied in mixed culture, the effects of must characteristics on the fermentation outcomes remained unknown.

Therefore, the impact of the main components of grape must, i.e. concentration of sugars, nitrogen and lipids, was studied in synthetic must thanks to a Box-Behnken experimental design. Non-*Saccharomyces* yeasts were tested in sequential inoculation with *S. cerevisiae*. The progress of the fermentations was monitored. Main metabolites and aroma compounds (higher alcohols, acetate and ethyl esters, acids and thiols) were measured at the end of the fermentation. Different aromatic profiles were obtained and the impact of each parameter as well as their interactions was assessed using the Box-Behnken model. Nitrogen appears to be the most influencing parameter. Results obtained in sequential inoculation were compared with those obtained in pure culture with *S. cerevisiae* to assess the benefits of non-*Saccharomyces* yeasts and to compare the differences in the response to must characteristics changes.

These results are of significance for the management of non-*Saccharomyces* yeasts in sequential inoculation for winemaking, and pave the way to more detailed metabolic and transcriptomic studies, in order to identify the metabolic and molecular basis underlying the phenotypic specificities of these species.

The study of hormonal metabolism of Trincadeira and Syrah cultivars indicates new roles of salicylic acid, jasmonates, ABA and IAA during grape ripening and upon infection with *Botrytis cinerea*

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Hormones play an important role in fruit ripening and in response to biotic stress. Nevertheless, analyses of hormonal profiling during plant development and defense are scarce. In this work, changes in hormonal metabolism in grapevine (*Vitis vinifera*) were compared between a susceptible (Trincadeira) and a resistant (Syrah) variety during grape ripening and upon infection with *Botrytis cinerea.* Infection of grapes with the necrotrophic pathogen *Botrytis cinerea* leads to significant economic losses worldwide.

Peppercorn-sized fruits were infected in the field and mock-treated and infected berries were collected at green, *veraison* and harvest stages for hormone analysis and targeted qPCR analysis of genes involved in hormonal metabolism and signaling.

Results indicate a substantial reprogramming of hormonal metabolism during grape ripening and in response to fungal attack. Syrah and Trincadeira presented differences in the metabolism of abscisic acid (ABA), indole-3-acetic acid (IAA) and jasmonates during grape ripening that may be connected to fruit quality. On the other hand, high basal levels of salicylic acid (SA), jasmonates and IAA at an early stage of ripening, together with activated SA, jasmonates and IAA signaling, likely regulate a fast defense response leading to grape resistance/ tolerance towards *B. cinerea.*

The balance among the different phytohormones seems to depend on the ripening stage and on the intra-specific genetic background and may be fundamental in providing resistance or susceptibility. In addition, this study indicated new roles for SA and IAA in defense against necrotrophic pathogens and gains insights into possible strategies for conventional breeding and/or gene editing aiming at improving grape quality and grape resistance against *Botrytis cinerea*.

Evaluation of the agronomic performance of cvs. Syrah and Tempranillo when grafted on a new series of rootstocks developed in Spain

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The choice of an adequate rootstock is a key tool to improve the performance of grapevine varieties in different '*terroirs*', as rootstocks confer adaptation to soil characteristics such as salinity, acidity, lime content or drought. Moreover, it is well-known that they also have a significant influence on the growth and vegetative cycle of the plants and, consequently, on yield and grape quality. Therefore, it is essential to have a sufficient supply of rootstock varieties in order that the winegrowers can choose the best suited to the different growing conditions. However, since the beginning of the 20th century, the development of new grapevine rootstocks has been very limited, despite growers' needs have changed dramatically.

The objective of this study was to evaluate the agronomic performance of cvs. Syrah and Tempranillo when grafted on nine new rootstocks belonging to the RG-Series, obtained by the Spanish nursery Vitis Navarra. The evaluation was performed in a 5-year-old vineyard located in Miranda de Arga (Navarra, Spain), where Syrah and Tempranillo are grown grafted on 21 different rootstocks (twelve commercial + nine new rootstocks). The vineyard was planted following a completely randomized experimental design, with three replicates of ten vines. Data were collected during four seasons (2014-2017). The new rootstocks significantly modified growth, yield, phenology and quality parameters in both varieties, some showing very promising features for higher yielding vineyards, and some not so productive but interesting for higher quality grape production.

Effects of climate change conditions (elevated CO_2, temperature and water scarcity) on phenology, physiology and grape quality of four Tempranillo somatic variants

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Environmental conditions are expected to vary as a consequence of climate change, being especially notorious the rise of atmospheric CO_2 levels, the increment of global temperature and the intensification of drought periods (IPCC, 2014). In the case of grapevine (*Vitis vinifera* L.), while the effects of these environmental factors have been studied independently, pointing towards changes in must composition (Salazar-Parra *et al.*, 2010), research on their interactions is important. Besides, the possibility of using different somatic variants for reducing undesired consequences of climate change is still to be fully understood (Carbonell-Bejerano *et al.*, 2015). In consequence, the objective of the experiment was to determine the effects of, on the one hand, an increment of atmospheric CO_2 and temperature and, on the other hand, water deficit (both combined and independently), on the physiology and berry composition of four different somatic variants of Tempranillo (306, 43, 1084 and VN31). Fruit-bearing cuttings of Tempranillo were grown in greenhouses under two temperature and CO_2 levels (24ºC/14ºC, day/night and 400 ppm CO_2 vs. 28ºC/18ºC and 700 ppm) combined with two water regimes (drought vs. well irrigated), from fruit set to maturity. The results show how differences based on environmental growth conditions affected plant phenology, leaf photosynthesis, plant growth, and berry composition. Tempranillo clones showed some differences in their response to these environmental factors.

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Drought stress in an arid region – influences on vine physiology, xylem anatomy and wine quality

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Abstract

Red grapevine varieties require a drought stress during specific phenological stages in order to achieve best wine quality. Yatir region has arid climate with low precipitation and high evaporation in summer, thus it's suitable for quality grapes growing. In recent years have appeared several problems, including leaf abscission, yield reduction, shrinkage of grape berries and more. The research aim was to explore the influence of drought stress on the grapevine vitality and vineyard suitability. We compared between SDI-Sustained deficit irrigation (grower's practice) and RDI- Regulated deficient irrigation with two weekly water amounts (irrigation model), while taking into account the leaf area and the changing climatic conditions.

Methods: Research was conducted from 2011 until 2015 on 7 ha vineyard planted with 3 red wine cultivars. The growing season was divided into three parts according to the phenological stages of the berry development: Stage I (from bloom to bunch closure), Stage II (from bunch closure to veraison) and Stage III (from veraison to harvest). Irrigation factors were appointed to each phenological stage and integrated into water amounts for each treatment. Evaporation was calculated by Penman-Monteith. Leaf area index integrated into the final model calculations. Various physiological parameters were measured: stem water potential at noon, LAI, gas exchange parameters, pruning weight and trunk diameter. Additionally, vessel diameters in the trunks, shoots and petioles were measured. Each repeat was separately used to produce wine, and then wine quality was tested by a qualified tasters panel.

Results: During Stage I (when the cambial activity is maximal) the vegetative growth was highest and water potentials were improved (less negative) under model irrigation as compared to growers practice treatment. Reduced irrigation during stages I and II caused more severe water stress in model treatment. Anatomical data showed that model irrigation increased vessel diameters in the trunk, wider annual rings and subsequently higher calculated hydraulic conductivity. In Cabernet Sauvignon and Shiraz yields were significantly higher under model irrigation. In Shiraz, however, the wine quality was similar between treatments, and in Cabernet Sauvignon the wine quality was better under grower's practice irrigation- though both were premium quality.

Conclusions: Water stress had a clear influence on leaf abscission, low yields and shrinkage of grape berries. We assume that wide xylem vessels combined with high LAI caused more severe water stress in model treatments during the stages II and III. However, grapevine varieties differed in their physiological response and wine quality. To conclude, for improved grapevine quality separate irrigation factors must be fitted for each variety and each phenological stage.

Characterization of grape varieties grown in the collection of Faculty of Agriculture in Belgrade (Serbia)

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Preservation of autochthonous grape varieties has been a priority in European countries since the end of 19th century to preserve from the extinction. Prospections of material have been traditionally done having into account local names and morphological characteristics. It has generated lots of synonyms and sometimes mistakes in the material preserved in collections. Molecular technics introduced for characterization during the last decades allowed an excellent identification of unique genotypes, synonyms and mistakes. Grape collection belonging to Faculty of Agriculture in Belgrade located at Radmilovac maintains accessions collected from Serbia. Thirty-two varieties were analysed using 26 microsatellites and morphological characterization was done using 48 OIV descriptors. Some of the varieties studied are currently cultivated in vineyards at different Serbian locations: *Smederevka* and *Zupljanka* are grown in large areas in the North and South of Serbia. *Prokupac* and *Plovdina* are Serbian varieties more represented in the vineyards of the South. *Beli Bakator*, *Veltlinac Crveni*, *Lipovina*, *Saperavi* and *Stanusina* are only grown in collections. *Bojadiser* and *Jagoda* are minor little-known varieties named so by their producers. *Godominka*, *Kladovska Bela* and *Jagodinka* are new created Serbian cultivars. All other cultivars are cultivated both in collection and on farm. A total of 27 different genotypes were found and six misnames were detected together with one colour mutant. Among unique genotypes there are four unique varieties not matching with other molecular profiles published in the bibliography and another one (*Jagoda*) found recently in old vineyards from Spain with other local name. These results confirm that a proper identification of material preserved in *ex situ* collections is required to put it in value and to evaluate it in the light of the new challenges (climatic change, market diversification). For this purpose, the joint use of ampelography and SSR markers remain highly reliable.

ICVV–SNP database: a tool for genetic studies in grapevine

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The ICVV has generated along more than ten years the ICVV-SNP database, made from the analysis of more than 10000 grapevine samples from all around the world: Algeria, Argentina, Armenia, Australia, Belgium, Bulgaria, Chile, France, Germany, Iran, Italy, Mexico, Montenegro, Morocco, Portugal, Romania, Slovenia, Spain and Tunisia. Along time, different technologies were used for generating SNP genotypes, but, unlike microsatellite markers, genotypes from a new technology are easily integrated in the database, needing no change or just a complementary base change.

Currently ICVV-SNP database contains 2866 different *Vitis vinifera* genotypes with DNA profiles for 48 nuclear SNPs. At present, 605 of them are considered as V.v. sylvestris and 2261 as *V.v. sativa*, 750 of which have a prime name supported by two independent evidences. Prime names are assigned according to the *Vitis* International Variety Catalogue (www.vivc.de), when possible, what provides access to additional information contained in this international catalogue. The 48-SNP-database alone is routinely used in our laboratory for cultivar identification, as these markers provide a very low probability of identity among grapevine cultivars.

In addition to the 48-SNP set, we use another set of 192 SNPs to generate extended DNA profiles. The resulting 240-SNP-database contains 1910 genotypes (about 1450 sativa). These 240-SNP profiles are used for pedigree studies, allowing the discovery of the genetic origin of hundreds of cultivars, including Tempranillo. Long SNP profiles also allow the analysis of genetic structure within the *Vitis vinifera* species and are useful in domestication studies, detecting feralized plants and crossing events between members of both subspecies.

Most genotypes in the database are also analysed for three chloroplast SNPs to establish their chlorotype. Grapevine chlorotype is maternally inherited and helps studying domestication and identifying the female parent in pedigree analyses.

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Integrating novel genotyping and phenotyping technologies for grape genetics

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Understanding the genetic determinism of crop yield, grape quality traits and adaptation potential is of paramount importance both for practical viticulture and for breeding new varieties to face future climate change challenges. The rapid development of advanced phenotyping and genotyping technologies will provide the information needed to cope with the efficient analysis of these complex traits by the use of relatively low-cost and robust systems. Here, we report the use of genotyping-by-sequencing (GBS, Keygene patents) to a large half-diallel grapevine population (N ~ 600) from 10 connected segregating progenies. The approach was useful to identify a total of 71,720 genetic polymorphisms, including bi-allelic SNPs and multiallelic markers. A subset of fully-informative and/or common markers to all (or 9) progenies were selected for the subsequent genetic grouping and mapping within each progeny. This process led to individual consensus maps with similar length than those previously published, but higher marker density. Individual maps were then merged to create a high-density grapevine integrated genetic map, with high informative content and acceptable marker order agreement between progenies. On the other hand, novel image-based phenotyping systems are designed for the automatic and non-destructive estimation of flower number, berry number and berry size, and available high-throughput phenotyping technologies are used to evaluate stress indicators of photosystems in selected individuals from the population. This novel genetic and phenotypic information will be integrated to identify QTLs and candidate genes involved in the genetic determinism of these relevant grapevine traits.

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Genetic and phenotypic characterization of grapevine natural variants for seed and fruit development

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Parthenocarpy and stenospermocarpy have been described as the two main mechanisms involved in the formation of seedless berries in *Vitis vinifera* L. Fruit development is usually induced by ovule fertilization, which triggers the growth and differentiation of the ovary and seed development synchronously[(1,2)]. In parthenocarpic conditions the ovary develops in the absence of fertilization yielding small berries that completely lack seeds, while in stenospermocarpy seed development aborts at an early stage after fertilization. Most cultivated seedless grapes exhibit the Sultanina-derived stenospermocarpy, which was recently associated to a missense mutation in *VvAGL11* gene[(3)], whereas other different sources of seedlessness have been less investigated[(4-6)]. Fruit set in the absence of fertilization ensures a good yield in unfavorable environmental conditions and in sterile genotypes, reasons why seedlessness is a key objective in genetic improvement programs of crops.

Our study focuses on several somatic variants for seed content discovered within grapevine germplasm collections and identified by SSR and SNP genotyping. Aiming to provide new insights into the mechanisms underlying seed and fruit development we evaluated fruit and seed set in open-pollinated, self-pollinated and emasculated conditions. The analysis of the ploidy level of seedlings obtained from occasional seeds of a seedless mutant and the microscopic observation of the gametophytes suggest the formation of unreduced gametes along with non-functional pollen grains and aberrant development of the embryo sac. RNA-Seq-based variant calling applied to the transcriptomic comparison of Sangiovese/Corinto Nero[(7)] allowed the identification of SNPs that were further confirmed in five cases. These polymorphisms may be applied for clone identification and are functional candidates for the seedless phenotype.

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Genomic, Morphologic and Oenologic characterization of Israel's grapevine population

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In the last 7 years our group is collecting and characterizing the Israeli grapevine germplasm. Our national survey effort resulted in the collection of more than 600 grapevines, both Vitis vinifera L. ssp. sylvestris and Vitis vinifera L. ssp. sativa. The ampelographic analysis shows clear differences between Sativa and Sylvestris groups in flower, leaf and cluster parameters, and that most Sativa belong to *proles orientalis*. Genetic population analysis was conducted by analyzing 22 SSR markers, determining first the unique genotypes. In addition, the population's structure was shown to have two distinct Sativa and Sylvestris populations, and a third mixed one. Likewise, the relationship between the Israeli grapevine population and sample grapevine populations in Europe and parts of Asia was investigated, showing that the Israeli Sativa and Sylvestris populations cluster closely together, suggesting a common genetic source. Next, 52 sativa and 8 sylvestris genotypes were sequenced using NGS approach. Population analysis of the Israeli collection, compared to European and Asian ones, using high quality SNPs, confirmed the common root of the Israeli sativa and sylvestris populations, as well as highlighted some specific genomic characterizations.

Finally, we produced wine by microvinification from 30 sativa and 5 sylvestris varieties. The wines were analyzed both chemically and organoleptically. 6 red sativa varieties produced wines containing alcohol, color, polyphenols and acid within acceptable oenological ranges, as well as 3 sylvestris lines, the last showing high color and polyphenolic levels. 10 white varieties showed proper alcohol and acid levels, and were aromatically profiled by SPME-GC-MS.

Two–source modelling of vineyard actual and potential (evapo)transpiration

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A robust assessment of crop water stress for irrigation schedule requires the estimation of both potential crop water needs and actual crop water use, defined respectively as potential and actual (evapo)transpiration. Potential evapotranspiration (ET_p) has usually been computed based on Penman-Monteith energy combination equation, which treats the land surface as a 'big leaf' composed of a mixture of soil and vegetation that is the single source for energy and water exchanges with the atmosphere. Furthermore, most applications consider that ET_p for a given crop is proportional to a reference rate ET_0 of a homogeneous and well irrigated short canopy such as alfalfa or grass through a crop coefficient K_c (i.e. $ET_p=K_cET_0$). However, vineyards show a unique vertical canopy structure and a strongly clumped plant distribution/row structure that could induce significantly different energy and aerodynamic regime compared to a homogeneous crop. Two-source models can account for clumped canopies with significant soil/substrate component by achieving a balance in the radiative and turbulent flux exchange with the lower atmosphere for the soil/substrate and vegetation elements, allowing hence for the partitioning between soil evaporation (E) and canopy transpiration (T). In this work we utilized modelling tools to evaluate the utility of one- and two-source models for predicting both potential and actual (evapo)transpiration. The models are applied to two years of continuous observations of eddy covariance fluxes, land surface temperature, canopy structure, and irrigation/rainfall, measured over two adjacent vineyards in California. When compared to ground ET measurements, results show a better overall performance of the two-source model versus single-source approach when estimating actual ET and T. Furthermore, modelling ET_p and T_p highlights the importance of a good parametrization of biophysical controls on maximum stomatal conductance, as well as how soil evaporation affects canopy transpiration through microadvection of heat and water vapour into the canopy layer.

The dried-fruit aromas of Merlot and Cabernet Sauvignon musts and young wines: impact of harvest date

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The intensity and complexity of aromas are very important in wine quality and play a great part in the global judgment of quality by consumers. Wine aromas take their diversity and complexity from various origins and cascades of formation including biochemical and chemical transformation or precursors found in grapes. For this reason, grape maturity can affect the flavor of wine. When grapes are harvested at maturity, the aromas found in young red wines of Merlot and Cabernet Sauvignon suggest a complex mixture of aromas of fresh red fruits such as cherry or blackberry for Merlot and strawberry or black currant for Cabernet Sauvignon. However, when grapes are harvested earlier, vegetal, herbaceous nuances dominate the flavors of these red wines, whatever the cultivar. On the contrary, grapes that are harvested belatedly, overripe, or have undergone a hot dry summer, produce very frequently a characteristic aroma of dried fruits (dried fig, prune). These aromas are found in both musts and young wines.

In order to investigate and identify specific molecular markers responsible for these nuances, organic extracts were prepared and analyzed by GC-O-MS. Furaneol (1), homofuraneol (2), γ-nonalactone (3), 3-methyl-2,4-nonanedione (4), (*Z*)-1,5-octadien-3-one (5), γ-decalactone (6), and massoia lactone (7) were detected at high concentrations in musts or wines marked by dried fruit aromas. Indeed, certain molecular markers of dried fruit aromas were specific to musts or wines. Reconstitution experiments revealed that a specific mixture of compounds (1-4) expressed dried fruit aromas in red wines. Grape dehydration, especially during exposure to white fluorescent light, increased the concentration of compounds (1-5) in M and CS must and wine samples. In addition, we report first results concerning the impact of harvest date on the sensory profile of the must and the wine associated with the formation of these chemical markers.

Improved methodology for early and accurate vineyard yield forecasting

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Vineyard yield forecasting is a key issue for vintage scheduling and optimization of winemaking operations [1]. High errors in yield forecasting can be found in the wine industry, mainly due to the high spatial variability present in vineyards and inadequate sampling methods [2]. Thus, improved methodologies for early and accurate vineyard yield forecasting are needed.

First, a study was conducted to: identify the main sources of spatial variability; and define an adequate sampling/measuring method. In Maule Valley (Chile, 2018), was addressed 23 vineyard blocks, comprising five cultivars (e.g. Cabernet Sauvignon) and four trellis systems (e.g. VSP). 268 experimental units were determined according to satellite image analyses. Multispectral and RGB cameras were used to obtain UAV aerial images and measure vine vigour (VV). Bunch sampling was carried out every two weeks for measuring Brix, bunch weight (BuW), berry weight (BW) and berry number (BN). Bunch counting was performed at fruit set, veraison and harvest, and BuW and BW evolution curves were built.

BuW was the main source of variability (CV=40%) and BW the least variable parameter (CV=20%), when comparing blocks and cultivars. Significant differences were found between veraison and harvest in BW, BuW and BuN, when analysing blocks. Cultivars and trellis systems with higher BuW showed lower BuN and higher Brix. Differences were found in VV when comparing cultivars, blocks and trellis systems (p-values below 0.05).

BuW and VV seem to be key variability sources that should be systematically measured. Mathematical models based on these data are being developed for yield forecasting. These models would be applied before veraison, to obtain an improved accuracy (error less than 15%).

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Effects of differing cultivation conditions on Crimson Seedless grapevine growth, physiology and yield water use efficiency

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The study examined grapevine performance as affected by differing cultivation conditions during critical phenological stages through direct plant-based measurements. The aim of this study was to determine the effects of differing soil texture classes and differing irrigation systems on Crimson Seedless table grapes physiology, yield, fruit quality and yield water use efficiency (WUE_y). Four blocks with different scenarios (A) drip on sandy clay loam; (B) micro-sprinkler on sandy clay loam; (C) micro-sprinkler on loamy fine sand and (D) drip on sandy clay loam were studied in the Hex River Valley over two seasons (2013/14 and 2014/15). No treatment was applied for this study and standard viticulture management practices as recommended for the production of export quality table grapes were applied in each block. Block A had poor vegetative growth, lower yield, as well as poor fruit quality in both seasons. While, Block D had the highest yield during the 2013/14 season, with the best fruit quality in both seasons. Higher values of net carbon assimilation rate and stomatal conductance corresponded with larger berry size and higher yield as observed in Block B, C and D. Whereas, high leaf temperature and vapour pressure deficit, combined with low Ψ_S reduced net carbon assimilation and stomatal conductance in Block A during the 2013/14 season

The two micro-sprinkler irrigated blocks had a tendency towards a higher WUE_y in the 2014/15 season, due to the higher evapotranspiration and yield measured in these blocks. The drip irrigated Block D had a higher WUE_{irr} in both seasons and produced grapes of the best quality. Thus, using a drip irrigation system and irrigation applications as applied for Block D and under similar conditions to that in this study, could reduce the volume of irrigation water used while maintaining best fruit quality.

Dynamic interaction between SO_2 and acetaldehyde during alcoholic fermentation

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In oenology, SO_2 is a major food additive; it is used from grape harvesting to the final stage of bottling for its various useful effects. The objective of this work is to investigate the impact of SO_2 addition on the fermentation process and on the yeast metabolism, particularly the production of aroma compounds. For that purpose, we studied the evolution of free and bound SO_2, but also the interaction between SO_2 and acetaldehyde to better understand the mechanisms that govern the production of these two key compounds during alcoholic fermentation. A lengthening of the lag phase duration was observed with the increase of the initial SO_2 content. But, depending on the initial level of SO_2, three behaviors occurred. From 0 to 30 mg/L, SO_2 production decreased with increasing of initial level of sulfites. At 30 mg/L, no variation of SO_2 concentration was observed. Beyond 30 mg/L, sulfite consumption was highlighted. Associated with these three physiological states, different ratios between free and bound acetaldehyde were measured and maximum accumulation of free acetaldehyde was observed at 30 mg/L of initial SO_2. Finally, production of aroma compounds was modified. Synthesis of compounds directly related to sulfur metabolism (such as methionol) was increased by sulfur complementation. On the opposite, production of molecules related to central carbon metabolism (such as α-acetolactate, isoamyl alcohol, isobutanol or 2,3-butanediol) was maximum at 30 mg/L of initial SO_2. These differences can be explained by intracellular redox perturbations arising from detoxification mechanisms used by yeast to diminish level of free SO_2 during the lag phase. Collected data clearly indicate that SO_2 additions impact not only the sulfur metabolism but also the overall yeast metabolism.

The challenge of quality in sulphur dioxide free wines: recent results

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Abstract

Sulphur dioxide (SO_2) is the most important and widely used preservative in winemaking due to its well-known antioxidant and antimicrobial properties. However, several human health risks, including dermatitis, urticaria, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated with its presence in wines.

The use of an excessive SO_2 doses must be avoided not only for health reasons but also because, from an oenological point of view, it can cause organoleptic alterations in the final product, neutralize the aroma and even produce characteristic aroma defects. Conversely, an insufficient concentration does not ensure the adequate stability of the wine against an excessive oxidation or microbial development, which can compromise its quality. Forbidding SO_2 as an antimicrobial agent without an alternative would increase the risk of wine spoiled by yeasts and bacteria. It is therefore important to continue the search for alternatives to SO_2 preservation to ensure a product that will comply with consumer demands with no health related problems. Nowadays consumers demand high quality foods that are free from additives, fresh tasting, microbiologically safe and with an extended shelf-life.

With all above in mind, researchers and the wine industry are looking for natural alternatives to replace SO_2 without significantly changing the quality and safety attributes of wine.

In the current presentation, the state of art on SO_2 wine replacements is thoroughly reviewed. Physical and chemical alternatives are counted and explained. Especial attention is focused in the use of polyphenols as a potential SO_2 alternative. The promising results of the use of grapevine shoots as alternative to SO_2 in wine are highlighted. Finally the current and future research on this topic is discussed.

Distribution of temperature, yeast cells and fermentation products in wine tanks during fermentations depending on the use of mechanical mixers during yeast inoculation, tank size and pillow-plate position

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Conditions in tanks are assumed to be homogenous due to agitation by CO_2 formation during vigorous phase of white wine fermentation. In pre- and post-fermentation phases, however, little CO_2 is formed presumably causing inhomogeneities. The objective of this work was to examine distribution of temperature, yeasts and fermentation products in wine tanks in different phases of the fermentation process. The impact of mechanical mixing during yeast inoculation, tank size (1-780 hL) and position of pillow plates was investigated.

Temperature distribution was measured by temperature loggers placed at different locations in tanks. Vertical gradients up to 8°C were revealed in the first days of fermentation when inoculation was performed through the bunghole without mixing. During this phase, flow cytometry showed high yeast counts in the upper part of tanks. Also high concentrations of ethanol, acetaldehyde, pyruvate and acetic acid, analysed by enzymatic tests, were found in upper parts. In lower parts, concentrations were considerably smaller. In conclusion, yeasts only sedimented slowly. After vigorous phase commenced, all parameters showed homogenous distributions.

When mechanical mixers were used during inoculation, fermentation started faster even in lower tank sections compared to inoculation without mixing. Independent from mixing, density-induced gradients of 0.5-1.5°C were observed depending on tank size in pre-fermentation phases. Distributions of yeasts and fermentation products were uniform for all depths. No gradients were present during vigorous phase.

When applying the cooling system after fermentation to retain CO_2 in the wines, cooling jackets placed in the lower half of tanks did not affect the upper half of the quiescent wine resulting in considerable temperature gradients.

The results provide valuable data on conditions in tanks indicating that sampling valve and temperature sensor not necessarily reflect the whole tank in pre- and post-fermentation phases. Moreover, mixing during inoculation shortens fermentation duration and increases control over the process.

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Wine grape quality assessment using hyperspectral imaging – a predictive analytics comparison framework

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The wine industry has been striving to achieve differentiation in their products and to improve their intrinsic quality and consistency. In this context, reliable and informative non-invasive in-situ approaches offer new perspectives to control the winemaking process and to boost the wine quality standards to a new level of excellence. Ensuring quality involves harvesting grapes at the optimal maturity point and selecting them according to the desired characteristics of the wine to be produced. In this context, hyperspectral imaging (HSI) combined with machine learning algorithms is a promising alternative to predict important enological parameters and assist on harvesting critical decisions. However, the large amount of data generated by HSI includes not only relevant but also a lot of redundant information that raise computational challenges for data-driven modelling. Several machine learning approaches have been proposed to handle such data characteristics, but selecting a suitable method is a cumbersome task and practitioners often rely on their preferred method, ignoring others that may bring predictive advantages. Therefore, in this work, we implement a predictive analytics comparison framework (PAC) to estimate relevant enological parameters for grape ripeness assessment. PAC covers the main classes of machine learning methods, namely, variable selection, latent variable, penalized regression, tree-based ensembles, and non-linear methods. The results obtained provide insights into the characteristics of the relationship between HSI of grapes and their enological parameters, providing guidelines for practitioners to identify the most promising approaches and pre-processing alternatives.

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Changes produced by the sulfur compounds in the release of polyfunctional mercaptans, as well as on the consumption of their precursors

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The volatile thiols 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexanol and 3-mercaptohexyl acetate are released during alcoholic fermentation from their precursors [1]. However, despite the knowledge of the metabolic pathways, there is not a direct correlation between precursor concentrations in musts and thiol concentrations in wines. Moreover, there are many factors that can influence the release of these compounds such as pre and post-fermentative operations. Sulfur plays an important role in many metabolisms, it is really important for yeast growth and fermentation metabolism, and can affect the production of many volatile compounds [2]. In this work, we determine the influence of different sulfur compounds on the release of polyfunctional mercaptans and on the consumption of their precursors. A set-up of fermentations of synthetic must containing known concentrations of polyfunctional mercaptans precursors was prepared. The effect of different sulfur compounds (elemental sulfur, sulfur dioxide, glutathione, methionine and cysteine) and the amino acids that are part of the glutathione (cysteine, glycine and glutamic acid) has been studied. Polyfunctional mercaptans and their precursors were analyzed in the final wine. Significant differences were found in the generation of these volatile thiols, as well as, in the consumption of their precursors when compared the different sulfur additions with a control without them. These results suggest that the consumption of these precursors is dependent on the must composition, and by modifying the sulfur source it is possible to change the release of these polyfunctional mercaptans. This could be a tool for wine industry to manipulate wine's aromatic profile.

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The impact of native beta–glucosidases on the aromatic composition of white and red wines

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The use of beta-glucosidases in the wine industry is potentially very interesting because they can promote the liberation of aromatic compounds from monoterpene or norisoprenoid-glycoside precursors. Since most Saccharomyces strains show no beta-glucosidase activity, the importance of non-Saccharomyces beta-glucosidases has been highlighted. So, the search of oenological yeasts that produce stable and active enzymes that contribute to the development of aromas in wine is a challenging area of research.

In order to explore the potential application of b-glucosidases to increase the varietal character of regional wines, we isolated beta-glucosidases from several oenological indigenous yeasts from Uruguayan vineyards. In this work, we present the results of two of the most promissory enzymes isolated from *Issatchenkia terricola* and *Issatchenkia orientalis*. In white Muscat wine, the treatment with *I. orientalis* -β-glucosidase did not show significant changes with respect to the control wine. Interestingly, the *I. terricola*-β-glucosidase increases the concentration of terpens (from 1143ug/L to 1743 ug/L) and norisoprenoids (from 0 ug/L to 100 ug/L) with respect control wine. Moreover, this enzyme showed higher specificity to norisprenoids than the commercial preparation. The high specificity of the *I. terricola* β-glucosidase for norisoprenoid glycosides, was also confirmed in Tannat wine, showing higher ability to liberate norisoprenoids and phenols from their precursors, than both of the other enzymes tested (*I. orientalis* enzyme and the commercial preparation). The sensory impact of these compounds on the aromatic properties of white and red wines was verified by a triangular test, resulting in wines with plum and raisins notes. This, together with the lack of activity on anthocyanin glycosides, highlights the potential of this enzyme in enology, since its high selectivity allowed the development of aroma without compromising wine color.

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Different Biosensor DNA-based platforms for wine authenticity: a comparative study

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The wine industry is a worldwide business dealing with an increasing number of economic interests. In order to guarantee the reliability of the bottled wine, products' follow a serious of traceability systems. Most of which are based on paper records which can be adulterated and/or mixed. There is a pressing need for analytical-based methods capable of inferring the grapevine composition and the geographical origin in a reliable way. During the last decades several innovative technologies have arisen dealing with both these problematics. In what grapevine composition is concerned it is unanimous that DNA-based technologies are the most adequate methods, since DNA is a stable molecule throughout the grape-wine chain. Within our group we have concentrated our research in developing a Long period grating (LGP) DNA-based biosensor suitable for *Vitis vinifera* L. varietal identification [1-3]. The aim of this work is to compare the LPG DNA-based biosensor with two other biosensor systems, based on nanoparticles and piezoelectric systems. Genomic DNA from leaf and wine samples was extracted using different grapevine varieties Malvasia Fina, Tinta Amarela and Tinta Roriz. The probes used were designed based on the SNPs found among the flavanone 3-hydroxylase gene [3]. Three independent replicates were performed for each assay. The three biosensor platforms were able to distinguish the grapevine varieties and were compared based on assay total time request, reproducibility and cost. These biosensors revealed the potential application of such systems for wine authenticity purposes, leading to their possible inclusion in a wide traceability protocol.

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Evaluation of *Saccharomyces pastorianus* impact to Sauvignon blanc chemical & sensory profile compared to different strains of *S. cerevisiae/ bayanus*

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White grapes of *cv.* Sauvignon Blanc were used to this study. 22 experimental fermentations (11 duplicated trials) were conducted by addition of different strains (& mixtures of them) & by using two different inoculation temperatures. The must was inoculated at cold conditions with a) mixture of S. *pastorianus* 1 (70%) / and S. baynus (commercial name BCS103 (30%), b) with a Mixture of S. *pastorianus* 2 (70%) / BS S103 (30%), c) with a S. *pastorianus* 1 alone, d) with S. *pastorianus* 2 alone, e) with BC S103 f) with CKS102 and g) with VL3. For Classic condition the must was inoculated with a) a mixture of S. *pastorianus* 1 (70%) / BCS103 (30%), b) with S. *pastorianus* 1, c) BCS103 and d) with VL3. Samples were taken for chemical and microbiological analysis from each bottle at 48 hours after the temperature inside the bottles reached at 18°C & at 2/3 of the fermentation for checking yeast implementation. The final wines were analysed for classical wine analysis, as also ethylic esters, superior alcohols, acetates and varietal thiols. Concerning the microbiological control the musts were evaluated for the yeast implementation by using PCR methodology at various levels of sugars consumption, in order to evaluate if no cross contamination has happened. Finally the wines were evaluated sensorially. Based on the data we could underline that for both *pastorianus* 1 and *pastorianus* 2 strains, the production of acetic acid is zero on cold conditions. Highest varietal thiols levels were recorded when using CKS and the mixed *pastorianus* 2 with BCS 103. Based on the sensory analysis results it was found that wines fermented with VL3 were evaluated as more intense on floral, citrus and herbaceous aromas, while CKS102 and *pastorianus* 2 were scored higher for tropical fruits while CKS102 and mixed *pastorianus* 2 higher for esters (fruity aromas).

Quality and safety control of wines by rapid on–site immunoanalytical methods

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The objective of the scientific activity of our group is the development of rapid, economic and user-friendly bioanalytical systems with applications in the area of food safety and quality. Analytical targets include varied compounds, like pesticides, hormones, additives, antibiotics, mycotoxins, phycotoxins, etc. In order to achieve this goal, we prepare synthetic functionalized derivatives and bioconjugates of the intended molecule and generate high-affinity monoclonal antibodies specific to the target compound. With these immunoreagents, we develop and validate immunochemical methods, including microplate-based immunoassays (ELISA), affinity columns, immunochromatographic strips, bioactive nanoparticles, and so on. These robust, affordable and versatile methods are particularly well suited for the on-site control in the agri-food industry, a sector with limited analytical resources but with a high volume of perishable samples. In this respect, in collaboration with a company leader in the control of postharvest fruit diseases, we have recently developed kits enabling food processors to carry out an effective and rapid control of fungicide residues by themselves, without needing an external analytical laboratory. This successful experience encouraged us to extend the application of these technologies to the wine sector. In a first example, results obtained on the use of immunoassays to the determination of a selected group of fungicides widely applied to fight grapevine diseases will be presented. Secondly, the performance of a simple, in-house developed antibody-based kit for the analysis of the mycotoxin ochratoxin A at levels well below regulatory limits in wine will be discussed. Finally, initial advances on the development of an ELISA test for the quantification of melatonin, a bioactive molecule whose presence in wine has been recently described, will be introduced.

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Application of UV-C light for preventing the light-struck taste in white wine

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The light-struck taste is a fault occurring in white wine bottled in clear bottles and exposed to light. The defect is due to the formation of methanthiol and dimethyl sulfide responsible for like-cabbage aroma arising from the reaction between riboflavin (RF), a highly light-sensitive compound, and methionine (Met). The light-struck taste is limited for RF concentration lower than 50 µg/L achieved through the choice of a *Saccharomyces* strain low RF-producer and the RF removal with charcoal and bentonite as fining agents [1]. Moreover, the protective effect of wood tannins has been recently showed, especially galla tannins [2].

Due to the RF sensibility to light, the UV-C light treatment was assayed. A synthetic wine solution spiked with RF (200 µg/L) and Met (3 mg/L) was irradiated with UV-C light up to 2000 J/L and RF decay was monitored. A linear decrease as UV-C light intensity increase was observed. RF was lower than 50 µg/L and 20 µg/L for 1500 J/L and 2000 J/L treatments, respectively. The addition of tannins (40 mg/L) led to a limited RF decrease (73%) maybe due to their shading properties [3].

Even though the UV-C light treatment is not admitted by the International Organization of Vine and Wine, its application could represent a tool for avoid the risk of light-struck taste development in bottled wine. The light exposure when the redox potential is high and the combined use of tannins could limit the appearance of this fault after the wine bottling preserving the wine quality during the shelf-life.

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Re-appearance of residual viable yeasts during wine microoxigenation applied after malolactic fermentation: Effect on the sensory profile of a Merlot wine

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Microoxygenation (Mox) applied after malolactic fermentation (MLF) aims at inducing an accelerated aging of the wine that allows an earlier release of the product to the market. Under ideal conditions, all oxygen ultimately reacts with SO_2, resulting in a stoichiometric mass ratio of 4:1, however in real conditions wines do not always obey to this ratio. It is hypothezised that after MLF, there are still some dormant, but biologically active microorganisms that can grow during Mox, and they would utilize this oxygen to increase acetaldehyde production. The aims of the present wtudy were to study 1) the effect of sterile filtration and SO_2 content on dissolved oxygen and yeast growth during Mox treatment, and 2) the effect of yeast development on color, acetaldehyde, anthocyanin-derivative pigments, tannins, sensory properties, and hedonic ratings. Sixteen sterilized 23-L-stainless steel tanks were employed. Half of them were submitted to Mox during 48 days at a rate of 15 mg L^{-1} $month^{-1}$ and half of them not treated (NMox). Four of the Mox and NMox tanks were microfiltered through 0.22 µm and four of them were unfiltered with the aim of evaluating the effect of residual viable yeasts on the treatments. The effect of the level of SO_2 was evaluated by adding two levels of free SO_2 (40 and 22 mg L^{-1}). Results show that the re-appearance of viable *S.cerevisiae* yeasts modulates oxygen and SO_2 consumption rates, inducing important chemical and sensory changes, particularly for aroma. Mox reduces green vegetable and reduction-related aromas, as well as in astringent mouth-feel properties related to tannin. Color changes during Mox are mainly dependent on the initial levels of SO_2. Preference among experts segregated into groups that prefer the cooked fruit flavors induced by Mox, and those who prefer the reductive spicy aromas in the no-Mox wines.

Oral Astringency: Influence of Chemical Species on Phenol–Protein Interaction

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Astringency is a tactile oral sensation that can be defined as a rough feeling as a result of exposure to substances such as alums or tannins. One of the most accepted mechanisms of astringency consist of the interaction between polyphenols and some specific salivary proteins (1). This interaction can be affected by different factors, such as the composition of the medium and the presence of other chemical species (2). For instance, the pH, the ionic strength or the presence of ethanol in the medium can influence this interaction since they can impact on the establishment of both hydrophobic interactions and H-bonds between the phenolic compounds and the proteins (3).

Based on these considerations, the present work aims to obtain further insights into the mechanisms in vitro and in vivo leading to a variation of astringency of polyphenols, evaluating the effect of the presence of different chemical species on the polyphenol-protein interaction using techniques such as SDS−Polyacrylamide gel electrophoresis (SDS-PAGE) and cell culture using a cell-based model of oral mucosa. Moreover, sensory evaluations have been performed in order to assess whether intervention with different chemical solutions can cause changes in the oral perception of wine astringency.

SDS-PAGE results showed a range of chemicals which seem to either enhance or inhibit polyphenol binding to salivary proteins. Changes in the salivary profile were observed by appearance and disappearance of protein bands. Cell culture assays indicated that oral cells were able to bind tannins, and this binding could be slightly inhibited in presence of some chemical solutions. The results obtained point out that polyphenol-saliva protein interactions are affected by some chemical species, what can help to better understand changes in astringency perception.

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Poster Session A

Molecular clues for the improvement of somatic embryogenesis in the grapevine

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Somatic embryogenesis is a biotechnological tool of interest for the grapevine, mainly due to the unicellular origin of the somatic embryos in this species. However, its application is still very empiric due to the lack of knowledge regarding the mechanisms controlling the formation and development of the somatic embryos.

One of the main limitations is the use of floral structures for somatic embryogenesis induction, being these structures only available a few days every year. In this work we analyzed the effect of inhibitors of histone deacetylases (HDACs) on the embryogenic competence of plant materials with different developmental degrees. The treatment with sodium butyrate 0.5 mM significantly increased embryogenic competence in cotyledonary embryos. In addition, this treatment produced the overexpression of HDACs-coding genes as well as of others related to embryogenic competence. These results open the possibility to obtain somatic embryos in the grapevine independently from the flowering season.

On the other hand, we have frequently observed precocious germination of somatic embryos during their maturation in the differentiation stage. This phenomenon restricts plant conversion of germinated embryos, thus reducing the efficiency of the protocol. The culture of the somatic embryo masses over a semi-permeable membrane on the differentiation culture medium produced a reduction of water availability for the somatic embryos; this allowed to significantly improve somatic embryo maturation, avoiding precocious germination. The analysis of the endogenous levels of ABA and polyamines and the expression of genes involved in their metabolism, revealed a complex regulation and interaction among them, overall driving to the accumulation of ABA as the key for the correct maturation of somatic embryos.

Biochemical foliar parameters baseline in pre-harvest status measured on *tempranillo* variety (La Mancha, Spain).

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Elemental content and biochemical parameters in pre-harvest status have their application for monitoring vineyard nutrition and the ensuing secondary metabolism pathways. Of these, biochemical analysis (antioxidant capacity, peroxidase, catalase, and phenols, carotenoids, flavonoids, anthocyanins, etc.) could be far more useful in determining the grapevines' status linked directly with the wine quality parameters. After many papers on this subject, a simple correlation between soil nutrient levels and grapevines' status has not had so far conclusive results. Among the reasons, we can include the wide variability of vineyard soil depth, physical conditions and biogeochemistry, differing vineyard design, cultural management and irrigation practices, diverse macro- and micro-climates of viticulture regions and vineyard sites. Moreover, the inherent differences in nutrient uptake and demand by grape varieties and rootstocks are also key factor to ascertain the above subjected purpose. The standard soil analysis could be useful in detecting the imbalances, or excesses, in certain chemical values and in problems related with the pH, salinity cation imbalances (magnesium: calcium: potassium).

Six zones were selected in the Subregion of La Mancha (Spain) called Campo de Calatrava with "t*empranillo*" variety grafted onto 110 Richter rootstock. Leaf samples were collected according to SCS-USDA guidelines (SCS-USDA 1972) at the beginning of ripening period (Last week of July 2017)) All samples were extracted and analyzed in triplicate. The trace and major elements content (soil, lea and grape) have been obtained by FRX techniques.

Biochemical and soil standard parameters were determined in the laboratories of ETSIA-UCLM.

From the results obtained in this study we can draw novel and relevant information about the behavior of vines and its relation with the environment. Moreover, baselines about the standard content of mineral elements and biochemical parameters are determined in order to stablish correlation between them and wine quality parameters.

Clone–dependent expression of grapevine esca disease

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Abstract

The occurrence of grapevine trunk diseases (GTDs) increases gradually within vineyards, leading to important economic losses. The worldwide cost of dead grapevines replacement is estimated over 1.5 billion dollars per year [1]. In France, close to 13% of the vineyard is affected [2]. Several factors could affect grapevine susceptibility to GTDs, mainly climate, vine age, soil fertilization, and also rootstock and variety [3]. Presently, no cultivars tolerant to GTDs could be identified and the level of disease expression for a cultivar can vary with region and from year to year [3]. In this context, our objective was to assess if the expression of esca disease, one of the most widespread GTDs, was clone-dependent.

Two clones (76 and 95) of the Chardonnay cultivar grown in the same plot were therefore compared according to their developmental and physiological traits, metabolome, and esca foliar symptom expression. Agronomical data were recorded during summer 2015, and metabolome analyses were performed in leaf samples collected from visually healthy vines as control (C), and from both symptomatic (D+) and asymptomatic (D-) shoots of diseased vines. GC-MS data showed that esca disease expression induces changes in leaf metabolome. Some metabolites were differently accumulated between C and D+ leaves for both clones. Moreover, differences in the metabolic profiles of C and D- leaves were only observed for clone 95. Finally, some metabolites accumulated in opposite ways in D+ leaves of both clones, with lower amounts for clone 76 and higher ones for clone 95. The complexity of these results highlights the difficulty of finding a common metabolic fingerprint associated with esca expression.

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To what extent do natural levels of ultraviolet radiation contribute to grape and wine quality?

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Ultraviolet (UV) radiation modulates the secondary metabolism in *Vitis vinifera* berry skin, which can affect the final composition of both grapes and wines. In this study, we evaluated the effects of ambient UV exclusion on the phenolic composition of both berry skins and the resulting wines, using Tempranillo grapevines grown under typical mid-altitude Mediterranean conditions. Grapevines were covered with either UV-blocking (FUV- treatment) or transmitting (FUV+) filters in a completely randomized block design, from flowering until harvest. Grapes were harvested at their optimal technological maturity and their respective wines were elaborated by microvinifications. Oenological characteristics of wines, together with the phenolic composition and antioxidant capacity of both berry skins and wines, were determined.

No oenological characteristic was significantly different between wines made of FUV+ or FUV- grapes. Regarding phenolic composition, the only phenolic group increasing significantly in FUV+ treatment, both in skins and wines, was the flavonols. Anthocyanins only showed significant differences between FUV+ and FUV- treatments in skins, and stilbenes only in wines, in both cases showing higher values in the FUV+ treatment. Hydroxycinnamic acids of skins showed a contrary tendency, with significantly higher values in the FUV- treatment. Hydroxybenzoic acids and flavanols were not affected by the UV treatment, neither in the skins nor in the wines. The total antioxidant capacity was significantly higher in the wine of the FUV+ than in the FUV- treatment, while in the skin, although the same tendency was observed, differences were not significant. In conclusion, natural UV levels in Mediterranean conditions seemed to be a factor of eustress ("good stress", defined as an activating and stimulating stress) for Tempranillo grapes, leading to a positive increase of the phenolic content and antioxidant capacity of the resulting wines. Thus, ambient UV radiation importantly contributes to the quality and healthy properties of wines.

Different physiological responses to water availability and its implication on water use efficiency in grapevine cultivars

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Under future climate-change conditions, the stomatal behavior can be a key issue, as a compromise between CO_2 adquisition and cooling. It has been reported that is not easy and correct to classify grapevine cultivars as strictly near iso-hydric or near-anisohydric. Moreover, the great variability observed in different studies indicates that a continuum exists in the range of stomatal sensitivities to water stress in grapevine. One of the shortcomings to study this is the different soil properties, rootstock used and/or environmental conditions of the studies used to collect the data. In the present study the physiological response to moderate drought of six non-grafted cultivars under the same environmental conditions were evaluated in pots, in order to measure how different response can affect plant water use efficiency (WUE) at different levels. Water use efficiency was determined at leaf and long-term WUE ($\Delta^{13}C$) level. Although all the cultivars were subjected to the same reduction on soil water content and all of them showed similar reductions in pre-dawn water potential, not all of them reduced stomatal conductance. Even the continuum between isohydric to anisohydric behaviors exists; we identified different strategies in response to moderate drought. Four cultivars reduced stomatal conductance under moderate drought, while in two cultivars (Escursac and Merlot) the reduction was not significant. The results corroborate the hypothesis that better stomatal control allows increasing leaf WUE under drought as occurred in Callet cv.; but Escursac and Merlot cv. showed high WUE under both treatments. In this case, high WUE can be related to maintain higher photosynthetic activity under drought. In addition, significant differences among cultivars were observed in chlorophyll content, osmotic adjustment and hydraulic conductivity. The different mechanisms involved in different strategies are discussed.

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Phytohormonal and metabolic reprogramming in grape berry (*Vitis vinifera* cv. Carignan) upon powdery mildew infection

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Grapevine (*Vitis vinifera* L.) is susceptible to several diseases; one of the most dramatic is powdery mildew (PM) that is caused by the obligate biotrophic fungus *Erysiphe necator*, which affects berries´ yield and wine quality. Phytohormones have been shown to play an important role in biotic stress response. In this study, metabolic changes and hormonal regulation upon PM infection of susceptible grapes is being analyzed. Naturally infected and control 'Carignan' samples were collected at green and *véraison* stages, and expression of genes involved in phytohormonal metabolism together with metabolic profiling was carried out in order to be integrated with future hormonome and transcriptome analysis. Genes associated with salicylic acid signaling pathway namely, *EDS1*, *PAD4,* and *PR1* were up-regulated in infected berries at both developmental stages. In what concerns ABA, the expression of *NCED* showed no significant difference between control and PM infected samples, though *ABA receptor PYL4* was more expressed in infected grapes at *véraison*. Concerning jasmonates, *MYC2* expression increased in PM infected berries at both stages, and *OPR1* showed a slight increase at the green stage. Regarding auxins, *IAA-amido synthetase GH3.2* and *AUX1* were highly expressed in infected berries at the green stage. These results indicate that both jasmonates and auxins may be important in defense response against PM in grapevine though these growth regulators are not classically related to defense against biotrophic pathogens.

Furthermore, metabolic profiling of control and infected berries revealed twenty-six metabolites that can be used as putative biomarkers of the infection at an early stage. These metabolites included several fatty acids, phenolic acids (gallic acid), phenylpropanoids (resveratrol and catechins), terpenoid precursors (tocopherol) and steroids (stigmasterol). Altogether, these data suggest an extensive hormonal and metabolic reprogramming upon infection with PM in particular at green stage.

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Arbuscular mycorrhizal symbiosis improved berry anthocyanins by modulating abscisic acid metabolism in Tempranillo (*Vitis vinifera* L.) grown under climate change scenarios

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Arbuscular mycorrhizal symbiosis is a promising tool for improving the quality of grapes under changing environments (Torres et al. 2016, 2018). Therefore, the aim of this research was to determine if the ability of arbuscular mycorrhizal fungi (AMF) to enhance phenolic content (specifically, anthocyanins) in a climate change framework could be mediated by alterations in berry ABA metabolism during ripening. The study was carried out on fruit-bearing cuttings of cv. Tempranillo (CL-1048 and CL-1089) inoculated (+M) or not (−M) with AMF. Two experimental designs were implemented. In the first experiment +M and -M plants were subjected to two temperatures (24/14°C or 28/18°C (day/night)) from fruit set to berry maturity. In the second experiment, +M and -M plants were subjected to two temperatures (24/14°C or 28/18°C (day/night)) combined with two irrigation regimes (late water deficit (LD) and full irrigation (FI)). At 28/18°C AMF contributed to an increase in berry anthocyanins and modulated ABA metabolism, leading to higher ABA-GE and 7'OH-ABA and lower phaseic acid (PA) in berries compared to –M plants. Under the most stressful scenario (LD and 28/18°C), at harvest +M plants exhibited higher berry anthocyanins and 7´OH-ABA and lower PA and dihydrophaseic acid (DPA) levels than –M plants. These findings highlight the involvement of ABA metabolism into the ability of AMF to improve some traits involved in the quality of grapes under global warming scenarios.

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Using image analysis to accurately determine the number of flowers per bunch for research purposes.

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Having a reliable method to get the number of flowers in a bunch is critical to analyze the effects of different stresses (biotic or abiotic) on blooming and fruit set. Here, it is presented a simple, yet, accurate method to obtain the number of flowers per bunch using a free software image analyzer. The bunches, are randomly selected at the experimental site and take it to the lab. In the lab, flowers are detached from the calyptra and placed in a black matt plastic sheet. Flowers are gently spread on the plastic sheet to avoid overlapping. Next, a picture is taken using a camera fixed to a holder. The plastic sheet must have a reference length for the further analysis with the software. The image is processed using the ImageJ software. Once processed, the number and perimeter of each flower are obtained. The bias analysis gives an overestimate of nearly 10 flowers, which, given the number of flowers per bunch, (for Tempranillo it could be more than 800) it can be considered an acceptable error. However, the bias can be diminished if a specific calibration is set. The images do not need to be clean. Non-desired elements can be removed from the analysis by applying a filtering method. The present methodology provides a simple, fast and precise technique to obtain the number of flowers in a bunch for research purposes.

Teinturier: to be or not to be–Alicante Bouschet *vs.* Vinhão/Sousón

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The color of grape berries is an important trait for the market acceptance and plays a significant role in determining wine colour. By definition a *teinturier* grape variety has pigmented skin and pulp. Nevertheless, the word "teinturier" comes from the French "to dye or stain". The Portuguese grape variety 'Vinhão' and its Spanish synonym 'Sousón' have been used in wine production since ancient times with the purpose of giving colour to wines.

In this study polyphenols were extracted from the skins and pulps of 'Alicante Bouschet' and 'Vinhão' grape berries, sampled at the ampelographic collection of the *Universidade de Trás-os Montes e Alto Douro* (UTAD) and of 'Sousón' sampled at the Viticulture and Enology Research Station from Galicia (EVEGA), at seven stages of berry development. Extracts were analyzed for total phenolic compounds, total anthocyanin and total tannin contents, and the anthocyanin profile was determined by High Performance Liquid Chromatography.

Berry skins of 'Vinhão' showed a higher content of total anthocyanins (17.0±2.5 mg/g of skin), than 'Alicante Bouschet' (14.3±1.7 mg/g) and 'Sousón' (12.7±1.2 mg/g), at harvest. As expected, pulps of the *teinturier* 'Alicante Bouschet' had a higher content of total anthocyanins (92.1±14.2 mg/L of pulp), in comparison to 'Vinhão' (14.1±0.7 mg/L) and Sousón (42.1±0.6 mg/L) varieties. Regarding phenolic content, skins and pulps of 'Alicante Bouschet' revealed a higher total phenolic content (71.2±8.0 mg/g of skin and 407.7±14.2 mg/L of pulp) than 'Vinhão' (61.9±8.9 mg/L of skin and 208.3±14.6 mg/L of pulp) and its synonym 'Sousón' (47.0±3.9 mg/L of skin and 350.4±7.2 mg/L of pulp). 'Vinhão' skins and pulps anthocyanin profile differed, suggesting that anthocyanin synthesis occurs also in the pulp cells and not merely migrate from the skins to the pulps.

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New technique to mitigate the impact of climatic change on the wine grape varieties

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The quality and typicity of wines, strongly depends on the quality of the grape cultivation. Actually, the increment in the average world temperature induces not only bigger irrigation necessities, but also earlier grape-ripening processes, which takes place in warmer days and shorter nights. This results in an imbalance between sugar and phenolic ripeness giving higher alcohol levels and less acidity in the must, which causes heavier wines with less liveliness and therefore less quality. According to Martínez de Toda et al. (2018 unpublished), we have been tested different forcing treatments in which the latent buds formed within the same year emerge after treatments were applied. This cause a delay of up to 1.5 months in the vine phenology stages, thus achieving later vintages where the temperature keeps fresher and giving rise to optimum grape ripening conditions. Three forcing treatments were carried out at stage H (separate floral buttons) D.O.Y. 115; at stage I (flowering) on D.O.Y. 135 and at stage J (fruit set) on D.O.Y. 145.

Forcing treatments carried out at stages G, I and J succeeded to delay ripening 18, 27 and 45 days respectively, compared to regular pruning. Vine yield was also significantly reduced by up to 79% resulting in high level of acidity in berries which was associated with a loss of flowers and/or a reduction in fruit set percentage. Moreover, a clear deregulation of the hormonal signaling was tested throughout the vine vegetative cycle during the forced regrowth from the newly emerged buds. A clear decline of citokinins was observed along the vine vegetative cycle but increased rapidly after pruning. However, ABA and Jasmonic acid showed an opposite pattern. Thus, from the formatted inflorescence, differentiation into flowers may be upregulated by citokinin. Moreover the absence of ABA may also promote the bud transition into flower.

Texture characteristics of grape berries during the botrytisation process

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Wine production from grape berries infected with *Botrytis cinerea* has hundreds of years of tradition in Tokaj region, in Hungary. The phenomenon, which leads to raisined "aszú" berries, is called noble rot, which is different from the well-known grey mould disease caused by the same fungus. It changes berries' properties resulting in high sugar concentration, complex aromas and different texture parameters of the grape berry. The botrytisation process was investigated according to two local varieties (Furmint and Hárslevelű) in the vintage of 2016. Berries were collected during ripening/rotting, distinguishing four infection phases (I. healthy berries, II. botrytised, not rotten berries, III. botrytised and rotten berries, IV. rotten berries with latent mycelia). Different compression tests and penetration tests were applied on whole berry and skin. For the testing a universal testing machine TAxT2i Texture Analyzer (Stable Micro System, Surrey, UK) equipped with a HDP/90 platform and a 30 kg load cell was used. According to the texture profiling analyses the berry- hardness, -cohesiveness, -gumminess, -springiness, -chewiness and –resilience were determined in all botrytisatin phases. The berry skin- brake force, -brake energy, thickness and Young's modulus of elasticity were measured in all phases by the penetration tests. Differences between the control berries and the second, third and fourth botrytisation phases were detected in the case of the most measured texture parameters. The measurement of berry hardness and berry skin thickness verified the softer texture characteristics of the second and third stages.

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Irrigation effect on nutrient evolution throughout the crop season for *Vitis vinifera* L. cv. Tempranillo blanco

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Nurititonal status could be strongly affected by water availability on soil throughout the crop season and, as a consecuence, it could affect to the final grape quality parameters.

The objective of this study was to determine the effect of drip irrigation on leaf blade and petiole nutrient evolution throughout the complete growing season. Sistematic monitoring study were carried out from a vineyard sited in Rincon de Soto (La Rioja; España).

The vineyard was planted in 2009 with the 'Tempranillo blanco' variety grafted on 110-Richter root-stock. Vine spacing was 2.6 x 1.1 m (3,500 vines/ha) and the pruning system is double Cordon Royat on a vertical shoot positioned trellis. Two irrigation strategies, with three repetitions each one, were carried out throughout 2013 and 2014:

CONTROL (C). Plots without supplementary water support.

IRRIGATION (R). Plots with supplementary water support by drip irrigation considering an irrigation strategy based on K_c = 0,3 from the start of the irrigation till *veraison*; K_c= 0,6 from veraison till harvest.

Blades and petioles were collected throughout the growing season and total N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B concentration were analyzed in both tissues. Furthermore, production components and grape juice quality parameters were also determined at harvest.

Effect of genotype and plant water status on the whole cluster respiration throughout ripening in grapevines (*Vitis vinifera* L.)

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An understanding of fruit respiration is critical to determining the carbon balance in grapevines. The effect of genotype and water status during the grape development was studied over two seasons (2013 and 2014) using a whole cluster respiration chamber. Whole cluster respiration were measured at hard-green, veraison and ripening stages under irrigated and non-irrigated conditions, and under light and dark conditions. Genotype influenced the fruit CO_2 efflux, that resulted in higher carbon losses in Tempranillo than in Garnacha. Plant water status appeared to have less influence on fruit respiration than genotype. Fruit respiration decreased from the first berry developmental stages to ripening stage. The fruit respiration rates measured using a whole cluster respiration chamber showed the photosynthesis and carbon losses. The study showed that genotype and plant water status play an important role in influencing carbon losses and fixation by fruit respiration under field conditions.

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Poster Session A

Water and soil management in vineyard: relationship between soil microbial community and its functional role with grapevine physiology and productivity

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In the last years the scientific community has been focused in the importance of soil biodiversity to improve crop sustainability; however, the effects of different agricultural techniques on microbial community and its functional traits are still unknown. The grapevine is an important crop worldwide, whose management has been changed over the years and the requirements of the moment. The use of cover crops for vineyard management has been largely studied in terms of agronomy and plant production. It is controversial due to competitive factor to nutritional and water resources, main limiting factors of that perennial crop. However, little is known about the effect of different management techniques on the microbial community and its functional activity dynamics and how it can affect plant water status and its final production.

In the present study the effects of permanent cover crop and tilled soil conditions under non irrigation and deficit irrigation, on soil activity and grapevine water status were assessed on vineyard. The study was performed in three phenological stages: flowering, veraison and harvest. Soil respiration, soil moisture and soil microbial functional diversity (using Biolog® Ecoplates) were analyzed in each phenological stage, as well as the plant stomatal conductance as indicator of its water status. During the harvest stage total grape production was assessed.

Soil activity (in terms of soil respiration and microbiota functional diversity) was affected by the plant phenology, and those effects were different in cover crop and tilled soil conditions. Despite total microbial functional diversity (Shannon index) was maintained in all the experiment, the degradation of different substrates by the soil microbiome of cover crop and tilled soil was observed. Grape production was also affected by both, soil and water management strategies.

These results highlight the above-underground dynamic relationship and how the agricultural practices can drive it.

A collaborative approach to determine when and how to measure grapevine water potential by means of a meta–analysis

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Water availability is acknowledged as the most limiting factor for vineyard productivity in arid and semi-arid climates. In addition, because of global change, water availability is becoming a key issue for vineyard performance in areas where, traditionally, little concern existed. In this context, irrigation necessarily plays (or will play) a major role to maintain vineyard productivity and wine quality. Worldwide researchers consider the Scholander pressure-bomb quick, flexible and accurate tool for the estimation of plant water status through the measurement of water potential (Ψ), a kind of "gold-standard" for water status determination. However, there are some discrepancies on how (stem vs. leaf) and at what time of the day (pre-dawn, morning, noon) measurements should be carried out to obtain the most reliable information.

In order to give an answer to those questions, thirteen research teams working in grapevine water relations throughout Spain compiled a complete dataset of water potential measured at irrigation experiments performed in vineyards. Researchers were requested to facilitate the original data (leaf by leaf) of water potential measurements for the implementation of a meta-analysis. Data were required to have been obtained in field experiments, comparing at least two doses of irrigation, and report at least five measurement days per year. The number of individual leaves measured and included in the meta-analysis was 78.854, comprising data from 438 experiments. Discrimination ratio (DR) was calculated for each experiment, and the obtained DR values compared. This comparison allowed determining that leaf water potential, either measured in the morning or at noon, provided much worse discrimination ability than pre-dawn and stem (morning or noon) water potential. Among the three latter, differences were much smaller, though morning stem water potential provided the best discrimination ability, followed by pre-dawn water potential. The results have practical implications for irrigation scheduling and for grapevine physiology studies.

Analysis of the physical composition of the berry, throughout the ripening period, in cv. Cabernet Sauvignon submitted to different water regimes, in the Duero valley

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Abstract. The water regime of cultivation affects the size and the composition of the berry, so that it acquires great importance with respect to the quality of the grape mainly in red varieties. The relationships between the different physical parameters of the berry and between these and some chemical or qualitative parameters can facilitate the understanding of the physical and chemical evolution of the grape, so it is interesting to know these relationships from measurements based in individual berries. The physical composition of the berry was studied throughout the ripening phase in 2014, through the components of its weight (total, pulp, skin and seeds), as well as the number and color of the seeds, and the concentration of sugars in the berry. The work was carried out in the Cabernet Sauvignon variety, through the cultivation of the vineyard submitted to the application of different water regimes, one of rainfed vines and the rest with moderate irrigation doses, in Valladolid (Spain). The application of irrigation slightly modified the weight of berry, as well as that of its components, and the concentration of sugars in the must, but the regression relationships between the parameters analyzed showed a remarkable variability, in general, in the different water regimes applied. The evidence of the observed relationships should serve to facilitate the decisions of water management of the vineyard as well as the criteria of execution of the maturity berry sampling during its evolution, mostly aimed at the determination of the harvest.

Regulation of night–time water use in grapevine: the effects of drought and vapour pressure deficit across genotypes

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Accumulating evidence indicates that stomata do not systematically close at night and that the resulting water loss by transpiration can be substantial (5-30% of daily water loss). This finding raises the question whether night-time transpiration (E_{night}) confers a functional benefit to the plant or is potentially deleterious resulting from incomplete stomatal control. In this study, we examined the variation of night-time transpiration among four different grapevine genotypes under well-watered and drought conditions and under a range of vapour pressure deficit (VPD). The potential correlation between night-time transpiration and leaf mortality was also examined. Four grapevine genotypes; Grenache, Syrah, 110Richter and Riparia Glorie de Montpellier were grown in 7 L pots and placed in a mini-lysimeter greenhouse platform that automatically measured soil water availability and whole plant transpiration rates in real-time. Drought treatment was induced by stopping irrigation and predawn, leaf, and stem water potentials were performed every 2-3 days on fully expanded leaves. Plant stomatal conductance was calculated from the transpiration values measured by the balances, the leaf and soil water potentials, and VPD (Charrier et al. 2018). No significant differences in stomatal control or E_{night} where evidenced between genotypes. However, in some cases night-time transpiration constituted a large percentage of daily water loss (as high as 50% of that of maximal day-time transpiration). Under well-watered conditions, E_{night} was positively correlated with VPD, but no correlation was observed under drought. The onset of leaf mortality differed significantly between genotypes. The link between water use regulation and leaf mortality in senescing leaves is discussed.

Whole–plant water use in field grown grapevine: seasonal and environmental effects on water and carbon balance

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Water scarcity is a main challenge in vineyards sustainability in most of the grapevine areas now and even more in near future to due climatic change perspectives. In consequence, water use efficiency measurements are of the highest interest to improve the sustainability of this crop. The vast majority of studies relays on measurements of carbon and water fluxes at the leaf scale. However, less data are available at the whole-plant scale.

We measured whole-plant water and carbon fluxes in grapevine exposed to two different water regimes at three phenological stages In September, measurements were repeated under high CO_2 to also check its effect at the whole-plant scale.

The results indicate that water and carbon fluxes are well coordinated between themselves under both water treatments. Under drought conditions, both fluxes were drastically reduced, but surprisingly the estimated water use efficiency was decreased, contrarily to what is shown at the leaf scale. The phenology also strongly decreased both water and carbon fluxes when compared to measurements in July. We hypostatized that harvest load respiration rates could have an important influence on whole-plant net carbon exchange (NCE). Finally, high CO_2 measurements, after correction for leaks, indicated an increase of whole-plant NCE as well as increased whole-plant water use efficiency, as expected.

Several technical issues were identified, like 1/ instability of [CO_2] during the night period that prevent robust estimation of whole-plant respiration and 2/ condensation during last night and sun-rise hours which may affect the estimation of daily plant transpiration.

Next studies should focus on the separation of the different plant organs and measurements of their respective weight in the whole-plant water and carbon balance through the season, as possible cause of the discrepancy between leaf and whole-plant water use efficiency estimations.

Diversity of microbiome of botrytised grape berries from Tokaj

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One of the most renowned dessert wines of the world is produced in the Tokaj wine region from botrytised berries. The phenomenon, which leads to raisined ‘‘aszú,, berries, is called noble rot which is different from the well-known grey mould and bunch rot diseases caused by the same fungus. Despite of many articles which pay attention to the determination of the suitable microclimatic conditions of botrytisation, the changes in the microbiome during the process has not been investigated in details. The aim of our study was to characterise the botrytisation process, focusing on the different stages of the development of disease and determine the microbial diversity. We investigated one specific vineyard in Tokaj region in Mád. For characterisation of botrytised grape berries two local varieties have been chosen that rot in a different way according to organoleptic observations. Berries were collected during ripening/rotting, distinguishing four infection phases (I. healthy berries, II. botrytised, not rotten berries, III. botrytised and rotten berries, IV. rotten berries with latent mycelia). Biodiversity of bacteria, yeast and filamentous fungi in all phases and cultivars were analysed by molecular biological methods. In order to get an overview about the microbiome populations the following sequences were analysed: 16S V4/V5 region; 18S D1/D2 region and the fungal ITS1/ITS2 region. In conclusion, the composition of the population of identified microorganisms showed variances in the different phases.

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Big Changes at Veraison: Acidity and Respiration

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Organic acids play an important role in the production of quality wines. The accumulated malate in grapes is consumed by respiration and gluconeogenesis processes throughout the fruit maturation phase. Respiration losses can be compensated in part by CO_2 fixation in the fruit. The current scenario of climate change with high temperatures causes a general reduction of malic acid in grapes during ripening, with potentially negative consequences for wine quality. However, many physiological changes occur at different times and rates in different berries on the same cluster or vine, as exemplified by the phenological stage of veraison. The main objective of this work was to study the variability of titratable acidity (TA), organic acids, respiration and photosynthesis in different berries on the same cluster at veraison, and to investigate the relationship between these parameters in this short period of time. An experiment was carried out in 2016 using field-grown Merlot vines. The changes in berry weight, soluble solids, TA, organic acids, respiration and photosynthesis were measured in berries at seven stages: hard green, soft green, pink, red, blue, ripe and overripe. The results showed the massive and rapid changes that occur in grape berries at the beginning of ripening, the variability of the measured parameters, the relationship between them, as well as the importance of understanding "veraison" considering each one of the stages that compose it.

Key words: malic acid, tartaric acid, titratable acidity, fruit gas exchange

Residues of pesticides and some of their degradation products in natural waters from vineyard areas located in Rioja Alavesa and Rioja Alta regions

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Pesticide consumption in La Rioja region (Spain) is very high, especially in vineyard areas, and data are scarce regarding the concentration of these compounds in surface and ground water. A related work carried out in 2010-2011 by this group highlighted the presence of several pesticides and some of their degradation products in natural waters from D.O.Ca. Rioja. The objective of this work was to evaluate the presence of the main compounds detected in that previous work and several new compounds. A multi-residue method was developed based on solid phase extraction (SPE) and liquid chromatography coupled to mass spectrometry to quantify the selected pesticides with detection limits lower than 0.1 μg L^{-1}, which is the limit established by current EU legislation for the determination of pesticides in drinking water. Screening and quantification of 60 compounds includeding 12 herbicides, 22 fungicides, 14 insecticides and 12 of their degradation products were carried out in 20 samples of surface and ground waters from an area included in the region of Rioja Alavesa and Rioja Alta. Samples were taken in September 2017 and January 2018 corresponding 13 of them to the highest polluted samples analysed in 2011. The analysis showed a decrease in the concentration and the number of samples contaminated with the herbicides terbuthylazine, its degradation product deethyl-terbuthylazine and fluometuron, especially in the samples collected in January. These compounds were the most ubiqous determined in the previous analysis. Results also highlighted the presence of high number of fungicides especially metalaxyl, its degradation products and tebuconazole, that were the compounds detected in the highest concentrations (79% of samples from Rioja Alta) and several of the new compounds included. However these concentrations were clearly lower than the concentration detected in 2011. One more time, insecticides were the compounds less present in analysed waters.

Entomopathogenic nematode natural distribution in vineyards managed with cover crops: impact on activity and soil organisms' assemblage

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Viticulture is a worldwide valuable sector, with special relevance in Spain for their socio-economic impact. Vineyards are threatened by numerous pests and diseases, and their management relies primarily on use of conventional agrochemicals. Current paradigm of sustainability pursues the implementation of ecologically sound strategies. The use of cover crops is arising as an alternative with numerous benefits, including favouring above-belowground biodiversity. We hypothesize that vineyard with cover crops might increase the prevalence of entomopathogenic nematodes (EPNs), biocontrol agents of soil pests, modulating the assemblage of other associated organisms. The presence and activity of native EPNs were evaluated in an ongoing experiment in La Grajera (Logroño, Spain) on *Vitis vinifera* var Tempranillo (clon RJ-26, rootstock "110-Richter") investigating tillage, and three types of covers: spontaneous, flower-driven, and seeded with *Bromus perenne* (Poaceae) (n = 3 per treatment). During late-spring and early-autumn 2017, 48 composite soil samples (12 cores, 2.5 cm diam X 20 cm depth) were taken to ensure balanced spatial distribution (two pair's rows/inter-rows per plot). Each sample was assessed for the presence and abundance of 9 EPNs and other related organisms: 6 nematophagous fungi, 5 free-living nematodes (FLNs), and 2 ectoparasitic bacteria, by qPCR approaches. Moreover, traditional insect-bait provided the measurement of EPN activity. In spring 2017, nematode activity was slightly higher ($P = 0.068$) in inter-rows than in rows. However, this trend was only observed for the tillage and seeded treatments in later autumn ($P < 0.05$). Two EPN species were isolated: *Steinernema feltiae* and, for the first time in La Rioja, *Heterorhabditis bacteriophora*. The insect-baits detected low EPN-FLN incidence in the isolates, indicating that EPNs could suffer low competition with other nematodes for the cadaver under this scenario. The ongoing surveys in 2018 will supply additional data on their natural distribution and assemblage.

Effects of the ground cover management on the phytoseiid mites abundance in a Mediterranean vineyard

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Conventional practices in vineyards increase its vulnerability against pests. Phytoseiid mites (Acari: Phytoseiidae) are phytophagous predators and an essential part of its Integrated Pest Management (IPM) programs. Some species of phytoseiid are specialized predators of tetranychid and eriophyid mites. In this study, we evaluate the impact of ground cover management (tillage, native cover crop and flowering cover crop) on phytoseiids abundance in a vineyard of La Rioja (Spain) from May to September and for two consecutive years (2016 and 2017).

The native cover crop was characterized by the presence of *Bromus tectorum*, *Capsella bursa-pastoris*, *Conyza* sp., *Galium aparine*, *Hordeum murinum*, *Melilotus* sp., *Stellaria media*, *Urtica* sp. and *Veronica hederifolia*. The flower cover was mostly composed by *Calendula officinalis*, *Centaurea cyanus*, *Cosmos bipinnatus* and *Eschscholzia californica*. Phytoseiids were sampled using vine leaves randomly taken and picking ground cover vegetacion for native and flowering cover crops.

The phytoseiid community was dominated by *Typhlodromus pyri*. Moreover, individuals of *Euseius stipulatus, Kampimodromus aberrans, Paraseiulus talbii, T. phialatus and T. recki* were captured.

Phytoseiid mites on leaves showed a significant increase in flowering cover crop at certain summer sampling dates. However, there are not significant differences on total abundance of phytoseiids on leaves among treatments. On the other hand, the presence of native cover crop significantly affected total abundance of phytoseiids within ground cover vegetacion.

Investigation of the role of the polysaccharide pullulan in the development of Esca disease of grapevine

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The Esca disease of grapevine belongs to the group of grapevine trunk diseases which cause great losses from year to year. The main pathogens of Esca disease (*Phaeomoniella chlamydospora*, *Togninia minima*) are known producers of the polysaccharide pullulan. According to the literature, this substance is probably participate in the development of Esca disease, however it's role is not completely clear. Here we present the results of our investigations on the possible synergistic interaction of pullulan with the secreted effector proteins of *P. chlamydospora* (Pch).

Microscopic investigation of the interaction of fluorescently labelled pullulan with onion cell suspension showed that the polysaccharide can establish a physical interaction with the cell membrane and suggested to be internalized into the host cells. The interaction of pullulan with Pch proteins was investigated by affinity electrophoresis. The results clearly showed that at least some portion of the secreted Pch proteins can bind pullulan. According to trypan blue absorption assays combined with spectrophotometry, the Pch proteins show increased toxicity against onion cells in the presence of pullulan. All the above results suggest that pullulan may aid the internalization of certain Pch proteins into the host cells by anchoring them to the plasma membrane.

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Effect on grape and wine quality of the ozonated water used in vineyards to control wood diseases

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Ozone is a powerful oxidant with a high capacity to fight against various types of microorganisms, especially fungi; in this process, ozone self-decomposes quickly and leaves no toxic residues. Wood diseases are one of the main problems of the vineyard that are caused by several types of pathogenic fungi, so it is interesting to know the effectiveness of ozonated water to combat this type of diseases. In the last three vintages we have studied how ozonated water treatment influences the recovery of grapevines affected by this disease. For this purpose, different ozonated water application strategies have been carried out over more than 1700 vines of different varieties of white and red cultivars from Castilla-La Mancha Spanish region, which had different degrees of development of the disease. The similarity of visual symptoms of treated plants affected by the disease with healthy or untreated plants was evaluated, and a different response was observed depending on the grape variety. Thus, more than 80% of the treated affected plants of the Cabernet Sauvignon variety showed similar appearance to the healthy plants. Among the other varieties, an increase of 13% of improved treated vines was observed in Tempranillo and of 4% in the rest of varieties compared to their respective controls. The quality of the grapes from the treated vines and their wines was evaluated considering the classic enological parameters as well as their phenolic and aromatic composition. The ozonated water treatments did not modify the development of the alcoholic fermentation, but affected the phenolic and aromatic content of the grapes and wines in a different way depending on the year of harvest, the application strategy and the variety of grapes.

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Predator insect community in a vineyard agroecosystem: influence of the surrounding vegetation

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Intensive agriculture and monoculture practices have caused landscape simplification and an accused loss of biodiversity, leading to a negative impact on the functionality of the agroecosystems and the ecosystemic services. The functional group of natural enemies has a vital role as they regulate the crop pest populations. Thus, using conservation strategies by managing agricultural habitat could play a significant role on the effectiveness of the ecosystemic service provided by natural enemies.

The aim of this study was to determinate the predator insect community in a vineyard agroecosystem to the family level, and to study the influence of surrounding vegetation as predator insect suppliers. Combi and pitfall traps were used to sampling the insects. The individuals were identified and data about abundance and diversity were obtained. Composition and structure of the predator insect community were studied by abundance models using the rank-abundance curve. To evaluate the influence of the vegetation, Shannon and Simpson diversity indexes were calculated. Besides the comparison between samples next to vegetation and samples away from vegetation was conducted for each predator insect family.

Predator insect community was dominated by Formicidae, followed by Coleoptera. In all the other predator families, lower abundances were obtained, especially for Geocoridae, Mantidae and Neuroptera predators. Biodiversity assessment showed no significant differences between groups near and far from vegetation, although slightly higher values were obtained on samples close to vegetation. Regarding to the influence of vegetation on each predator family, although no significant differences were observed, abundances were higher on the group near to vegetation (except for Anthocoridae, Aeolothripidae and Formicidae).

A web-based decision support system for the sustainable management of vineyards

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The transition from conventional to sustainable viticulture requires increased knowledge of the farming system and makes decision making for crop management complex (Rossi et al., 2012). In Europe, the framework for sustainable viticulture is the Directive 128/2009/EC on the Sustainable Use of Pesticides that makes integrated pest management (IPM) mandatory across Europe. IPM promotes low pesticide control of harmful organisms based on crop monitoring and tools for decision-making. A decision support system (DSS), called vite.net, was developed for vineyards, powdered by Horta, a spin-off of UNICATT. The DSS is a web-based tool able to: i) collect multiple information/data in real-time about different vineyard components (air, soil, plants, pests, and diseases) by using IoT technologies; ii) analyze these data by advanced modelling and bigdata solutions; and iii) make up-to-date information, alerts and decision supports for vineyard management. The DSS considers grapevine growth and development, risk for diseases (downy and powdery mildews, black-rot and Botrytis bunch rot) and pests (berry moth, American leafhopper and mealybugs), protection dynamics of fungicide applications, and abiotic stresses (drought, low and high temperatures). In season 2017, the DSS was used by 300 farmers on approximately 12.000 ha across Italy and in 36 pilot vineyards in Spain. The use of the DSSs significantly increase the farmer's profit and decrease the environmental impact of crop production.

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Exploit biodiversity in viticultural systems to reduce pest damage and pesticide use, and increase ecosystems services provision: the BIOVINE Project

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Organic vineyards still rely on large external inputs to control harmful organisms (i.e., pests). The BIOVINE project aims to develop natural solutions based on plant diversity to control pests and reduce pesticide dependence. The capability of plants of increasing the ecosystem resistance to pests and invasive species is a well-known ecosystem service. However, monocultures (including vineyards) do not exploit the potential of plant diversity. BIOVINE aims to develop new viticultural systems based on increased plant diversity within (e.g., cover crops) and/or around (e.g., hedges, vegetation spots, edgings) vineyards by planting selected plant species for the control of arthropods, soil-borne pests (oomycetes, fungi, nematodes), and foliar pathogens. Candidate plants will be identified by a literature review, and the selected ones will be tested in controlled environment or small-scale experiments. The ability of the selected plants to: i) attract or repel target arthropod pests; ii) conserve/promote beneficials; iii) control soil-borne pests by means of biofumigation; iv) carry mycorrhizal fungi to the vine root system to increase plant health (growth and resistance); and v) control foliar pathogens by reducing the inoculum spread from soil, will be investigated. New viticultural systems able to exploit plant diversity will then be designed based on results of BIOVINE activities, following a design-assessment-adjustment cycle, which will then be tested by in-vineyard experiments in France, Italy, Romania, Slovenia, Spain and Switzerland for a 2-year period. Innovative viticultural systems should represent an improved way for pest control in organic viticulture, meanwhile they should positively affect functional biodiversity and ecosystem services. New control strategies may provide financial opportunities to vine growers and lower their reliance on pesticides.

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Droplet digital PCR: an innovative technology for *Cadophora luteo-olivacea* quantification in grapevine planting material

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Cadophora luteo-olivacea is currently considered one of the main fungal trunk pathogens associated with Petri disease of grapevine in many production areas worldwide. In the present work we do advocate the use of the most innovative detection tools available, such as the droplet digital PCR (ddPCR), which could lead us to a more suitable management of these trunk pathogens. The advantages of this high-throughput technology over other quantitative technologies such as real time PCR are the possibility for absolute quantification without the need of standards, the insignificance of primer efficiencies and the increased assay sensitivity due to dilution of PCR inhibitors in droplets. In this study, a rapid and sensitive TaqMan assay was developed for ddPCR to quantify *C. luteo-olivacea* in grapevine planting material. DdPCR performance was evaluated using DNA of 1296 grapevine grafted plants ready to be established in the field. *C. luteo-olivacea* was detected in 95.4% of analysed plants; nevertheless, the quantification of the fungus was widely variable: 3.0% of the plants presented more than 1000 copies/µl, 27.3% between 1001-100 copies/µl and, 65.1% between 99-4 copies/µl. Only 4.5% of the analysed plants were considered as healthy plants (less than 3 copies of the fungus per µl). The present work has showed the potential of the ddPCR technology to quantify *C. luteo-olivacea* in grapevine plants. Further research is needed to determine minimum infection thresholds needed for *C. luteo-olivacea* to start infection in the field and to understand which planting conditions will enhance disease proliferation and benefit pathogen colonization.

Germplasm screening of Tempranillo clones for resistance to *Neofusicoccum parvum*

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Botryosphaeria dieback is a grapevine trunk disease caused by a number of Botryosphaeriaceae spp., being *Neofusicoccum parvum* the most virulent species associated with this disease. To date, no curative measures are known for control of GTDs; therefore, planting disease-resistant cultivars is a time-tested and sustainable approach for disease control. The aim of this study was to identify sources of resistance to *N. parvum* among the Tempranillo germplasm collection of the ICVV in Spain. For this purpose, 47 Tempranillo clones were selected based on the visual assessment of symptom expression in standing vines over the last 3 years, and inoculated with *N. parvum* strain BV-056 in a detached cutting assay under greenhouse conditions over two consecutive years. Five months after inoculation, cuttings were collected and inspected for lesion length. In addition, to investigate the induction of defense genes expression in grapevines, leaves from control and inoculated plants were studied at different periods after inoculation. The severity of internal wood symptoms caused by *N. parvum* varied considerably amongst the Tempranillo clones. No foliar symptoms were observed during the experiment. The most susceptible clone and the most tolerant one were selected for real-time RT PCR analysis of relative gene expression. Our research could help to explain why the clones manifested disparate tolerance to the infection caused by *N. parvum*.

Efficacy of white mustard biofumigation to reduce fungal infection and inoculum density of black–foot disease pathogens in vineyards

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The effect of white mustard cover crop residue amendment and chemical fumigation with propamocarb + fosetyl-Al combined with *Trichoderma* spp. root treatment was investigated for the control of black-foot disease of grapevine in two commercial fields under grapevine nursery planting material rotation. Treatments were a factorial combination of three main plots (no soil treatment control, soil amended with white mustard residue, and soil chemical fumigation) and two subplots (application of *Trichoderma* spp. into the roots or untreated control). Inoculum densities of black-foot were measured before and after soil treatments as well as disease incidence, disease severity, and root mass. A total of 876 black-foot pathogens isolates were collected from grafted plants and grapevine seedlings used as bait plants in both fields. White mustard biofumigation reduced inoculum of *Dactylonectria torresensis* and the incidence and severity of black-foot of grapevine, but no added benefit was obtained when biofumigation was used with *Trichoderma* spp. root treatments. The effect of white mustard residues and chemical fumigation on populations of *D. torresensis* propagules in soil was inconsistent, possibly due to varying pretreatment inoculum levels. The results of our research showed that biofumigation with white mustard plants had potential for improving control of black-foot disease in grapevines. These control strategies can reduce soil inoculum levels and protect young plants from infection, providing grape growers and nursery propagators with more tools for developing integrated and sustainable control systems.

Phenotyping assays to identify tolerant grapevine cultivars to *Phaeomoniella chlamydospora* infection

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Grapevine trunk diseases (GTDs) are a significant threat to viticulture worldwide. Esca is one of the most destructive trunk diseases which is caused by a complex of fungal species, being the Ascomycete *Phaeomoniella chlamydospora* the most frequently isolated species from affected vines. To date, no curative measures are known for control of GTDs; therefore, planting disease-resistant cultivars is a time-tested and sustainable approach for disease control. The aim of this study was to identify sources of resistance to *P. chlamydospora* among the minority and commercial grapevine germplasm collections of the ITACyL and EVEGA in Spain. For this purpose, 15 and 41 cultivars from the ITACyL and EVEGA collections, respectively, were selected based on the visual assessment of symptom expression in standing vines over the last 3 years, and inoculated with *P. chlamydospora* strain BV-130 in a detached cutting assay under greenhouse conditions over two consecutive years. Seven months after inoculation, cuttings were collected and inspected for lesion length. The severity of internal wood symptoms caused by *P. chlamydospora* varied considerably amongst the cultivars. No foliar symptoms were observed during the experiment. The induction of defense gene expression in grapevine will be studied with the most susceptible and the most tolerant cultivars. Our research will help to explain why the cultivars manifested disparate tolerance to the infection caused by *P. chlamydospora*.

Interspecific adaptation of physiological P–days model to predict powdery mildew development threat periods

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Background: An adaptation of physiological P-days model was conducted to be applied as a predictive tool for *Uncinula necator* infections in a Ribeiro DO vineyard. We developed an integrated powdery mildew management strategy based on aerobiological, phenological and meteorological analysis to identify principal risk moments of disease development, which allows a phytosanitary reduction and leads to an optimization on chemical products use. This kind of research produces several economic and environmental profits, bringing closer agricultural practices to a sustainable system.

Methods: The study was carried out from 2008 to 2016, and 2017 data was used to validate the obtained regression model. *U. necator* spores were trapped using a Lanzoni VPPS-2000 and phenological observations were done on 22 selected plants of Treixadura cv. following the BBCH standardized scale.

Results: The highest total fungal spore amount was detected in 2016, with 16915 spores in the air of the vineyard, while the lowest amount was recorded in 2009, with 844 spores. In general, we found that highest daily spores concentrations were detected during Flowering stage (stage 6), during Inflorescence Emerge stage (stage 5) and Development of Fruits stage (stage 7). We modified P-days model by using cardinal temperatures for *U. necator* development and without accumulation of calculated values. Adapted equation was applied for all studied periods but we measure it prediction ability only for First and Maximum Peak values of spore atmospheric concentration within each considered year. For Maximum Peak we found that in general a 100 P-days value is exceeded, and we observed that for most of years P-days for 1-7 days before for Maximum Peak exceed a 150 value. In addition we observe a trend that can be defined as maximum P-days value obtained for the previous day of considered peak value, what happened in 55,5 % of cases (5/9) for Maximum Peak and 33,3 % of cases (3/9) for First Peak. A *t*-test was applied and no significant difference was found between real and forecast concentrations in 2017.

Conclusions: This results and graphical representation of spore levels and predicted P-days values show the usefulness of this model for fungal development prediction purpose. The conjunction of aerobiological, phenological and meteorological data supposes a great tool for disease development risk evaluation.

Cadophora luteo-olivacea: endophytic fungus or grapevine trunk pathogen?

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Cadophora luteo-olivacea has been considered during decades as endophytic fungus. It has been isolated from grapevine trunk, soil samples and also from extreme environments as the Antarctic continent. During a three-year survey conducted in Ribera de Duero (Spain), the percentage of *C. luteo-olivacea* detection increased notably, although it was detected from symptomatic as well as from asymptomatic plants.

Accordingly, the objectives of this study were the detection of *C. luteo-olivacea* from nursery plants, field plants and soil samples; to analyse the pathogenicity of different *C. luteo-olivacea* isolates, and finally to test natural compounds and biocontrol agents able to fight against *C. luteo-olivacea*.

The results suggest that *C. luteo-olivacea* could be considered as pathogenic fungus because of different isolates were able to produce necrotic lesions in inoculated plants. Finally, we propose two different eco-friendly treatments, first one based on plant extracts and the second one based on biocontrol agents. Both treatments were able to reduced significantly the *C. luteo-olivacea* growth being a promising tool for managing grapevine trunk diseases.

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In Vitro evaluation of bio-control agents against fungal pathogens causing grapevine trunk diseases

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To date, up to 133 fungal species belonging to 34 genera have been associated with Grapevine trunk diseases (GTD). GTDs are primarily caused by ascomyceteous fungi but some basideomiceteous taxa are also thought to play an important role in this disease complex (Gramaje *et al*. 2018). This research is aiming to screen biocontrol agents conjunctively for controlling Esca, Petri disease, Eutypa dieback, and Botryosphaeria dieback.

-Among the different fungi occurring in Esca and Petri diseased vines, *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* are the most prevalent.

-Eutypa dieback is caused by 24 species in the Diatrypaceae the most virulent and common of which is *Eutypa lata*.

-To date, 26 botryosphaeriaceaous taxa have been associated with Botryosphaeria dieback of grapevines. Pathogenicity studies have demonstrated that species within the botryosphaeriaceae family, *Neofusicoccum parvum* are among the fastest wood-colonizing fungi and hence the most virulent GTD fungi.

This study characterized the biocontrol potential of cultivable fungal and bacterial isolates. Genomic DNA of the selected bacterial and fungal isolates was extracted and partial characterization of 16S rRNA and ITS performed respectively. A dual culture assay was used for the detection of their antimicrobial activity against the studied plant pathogens.

This study therefore provides baseline information on the potential biocontrol microbes against *P. chlamydospora*, *Pm. minimum, E. lata* and *N. parvum* that could be used in the management of these diseases.

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Bibliography: Gramaje, D., Úrbez-Torres, J. R., & Sosnowski, M. R. (2018). Managing Grapevine Trunk Diseases With Respect to Etiology and Epidemiology: Current Strategies and Future Prospects. Plant Disease, 102(1), 12-39.

Modulating effect of green fertilizers on heavy metal toxicity

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Heavy metal pollution is one the most important environmental problems in marine, terrestrial, and freshwater areas. This study evaluates the modulating effect of different commercialized green-fertilizers on the toxicity of cadmium (Cd) to plant germination. Experiments were carried out in hydroponic systems, using three day-germinated lettuce seeds. Effects of the green-fertilizers (with or without cadmium) were assessed by measuring the hypocotyl growth after four days of treatment. The Cd concentration was selected as the EC50 (32.3 µM) calculated from dose-response experiments assessing the toxicity of Cd on hypocotyl elongation. Four fertilizers were tested to know the concentrations leading to a growth improvement, although only AgroKaP-Kalibre at 0.01 and 0.1% had a positive effect on the growth (60% improvement). Furthermore, this product shown a dose-dependent protective effect against the Cd toxicity, and it was statistically significant when AgroKaP-Kalibre was used at 0.5%. The results proved the efficiency of this bioactive product in alleviating Cd toxicity in plant seed development, being potentially useful to decrease ecological problems caused by heavy metals in different contaminated environments including grapevine culture.

In Vitro evaluation of plant extracts against fungal pathogens causing grapevine trunk diseases

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Fungi are considered the most damaging microorganisms in agriculture and fungal trunk diseases are some of the most destructive diseases of grapevine in all grape growing areas of the world (Gramaje *et al.* 2018).

Grapevine trunk diseases (GTDs) would be best managed by an integrated disease management strategy. Due to the indiscriminate use of chemical treatments in agricultural products causes the development of pest resistance and affects human health. An alternative to synthetic fungicides is the use of natural products such as plant extracts for the management of fungal diseases in plants. In this sense, extracts from diverse plants: *Nerium oleander, Prunus dulcis* var. amara, *Quercus suber, Vitis vinifera*, *Humulus lupulus, Laurus nobilis, Rubus ulmiformis, Ruta angustifolia, Lavandula stoechas, Lupinus angustifolius, Lupinus luteus Salix angustifolia, Olea europea* were obtained. Thereafter, the antioxidant activity of each extract was measured, and the antifungal activity *in vitro* of extracts was evaluated against *P. chlamydospora*, *Pm. minimum*, *N. parvum* and *E. lata.* Based on an initial screening of 13 plant extracts, the most active treatments will be selected for further investigation.

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Bibliography: Gramaje, D., Úrbez-Torres, J. R., & Sosnowski, M. R. (2018). Managing Grapevine Trunk Diseases With Respect to Etiology and Epidemiology: Current Strategies and Future Prospects. Plant Disease, 102(1), 12-39.

In Vitro evaluation of plant extracts and bio-control agents against *Diplodia seriata*

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Diseases caused by species of Botryosphaeriaceae lead to significant losses of grape yield. *D. seriata* alone have been reported as being responsible for losses of up to 20% in the Bordeaux wine region of France (Larignon *et al*., 2001). Presently no curative measures are known for control of Botryosphaeria dieback in nurseries and young vineyards while in mature vines remedial surgery has shown to control the disease (Gramaje *et al*. 2018).

An experiment was conducted to test the efficacy of four plant extracts (*Nerium oleander*, *Quercus suber*, *Prunus dulcis* var. amara and *Vitis vinifera*) and eight bio-control agents, three strains of *Trichoderma* sp. (Two strains of *Trichoderma harzianum* and one strain of *Trichoderma virens)*, three isolates of *Clonostachys rosea*, and two isolates of *Penicillium chrysogenum*) in controlling *Diplodia seriata* under *in vitro* condition.

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Evaluation of plant growth–promoting rhizobacteria for bio–control of *Diplodia seriata*

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Plant-associated bacteria reside in the rhizosphere, phyllosphere, and inside tissues of healthy plants. A study was designed to screen individual strains of plant growth-promoting rhizobacteria (PGPR) for disease suppression *in vitro* and *in planta*.

Two bacterial strains, strain S1 and strain S2 produced five traits reported to be related to plant growth promotion, including nitrogen fixation, phosphate solubilization, indole-3-acetic acid production, siderophore production, and biofilm formation. Both strains were selected to assess their ability to protect grapevines from *Diplodia seriata* attacks, a pathogenic fungus commonly associated with Grapevine Trunk Diseases (GTDs).

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Metabolomic alterations in elicitor treated grapevine leaves monitored by ^{1}H NMR

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Pest control represents a predominant issue of viticulture and it is currently achieved by intensive use of fungicides. In response to the harmfulness of these chemicals on environment and human health, the development of sustainable disease control approaches is prevalent. During the past decade, the stimulation of defense responses by elicitors has become one of the most promising alternative strategy of plant protection as it is based on biodegradable substances, and provides long-lasting and wide-spectrum resistance. In this context, knowledge of impact of such compounds on grapevine primary metabolism is fundamental. The aim of this project was a metabolic characterization of grapevine leaves elicited by different molecules triggering jasmonic acid- and/or salicylic acid-dependent responses. The leaves of greenhouse *Vitis vinifera* L. cuttings were treated with methyl jasmonate (MeJA) and two commercial products: benzothiadiazole (BTH, Syngenta) and potassium phosphonate (LBG, De Sangosse). The global changes of metabolites under each condition in regard to untreated leaves were evaluated using the proton nuclear magnetic resonance spectroscopy (^{1}H NMR) followed by principal component analysis (PCA). The study showed the extensive reprogramming of primary metabolic pathways. The highest concentration of the majority of the identified metabolites, particularly sugars (*myo*-inositol, fructose, α-glucose, β-glucose and sucrose), some organic acids (malic, pyruvic, tartaric, ascorbic and fumaric acids), and phenolics (quercetin-3-O-glucoside, syringic, gallic and shikimic acids) was detected in control leaves. Some specificity according to the type of applied elicitor was noticed, but some common features also. Taken together, the most important changes were linked to carbon/nitrogen signalling which along with noticeably active Krebs cycle and decrease of key molecules in phenylpropanoid pathway suggest deviation of carbon and energy from primary to secondary metabolism in stress mimicked leaves. Alterations in primary metabolism caused by studied elicitors highlight a necessity of further investigation.

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Suceptibility of cv. Tempranillo grapevines to the wood infection by *Diplodia seriata* and *Phaeoacremonium minimum*

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The fungal trunk pathogens *Diplodia seriata* and *Phaeoacremonium minimum* are involved in grapevine trunk diseaases (GTDs) such as Botryosphaeria dieback, Esca and Petri disease. Productivity of *Vitis vinifera* is reduced by GTDs in all grape-growing regions of the world. The cultivar "Tempranillo" that are widely cultivated in Spain (201.051 Ha) is known as very susceptible to GTDs. The aim of this study was to evaluate a collection of 40 "Tempranillo" clones for susceptibility to infection by *D. seriata* and *P. minimum*. Experiments were conducted on rooted, 1-yearold dormant cuttings of forty "Tempranillo" clones. The susceptibility of the clones was assessed by inoculating the potted cuttings in the greenhouse with a mycelium agar plug of pure cultures of *D. seriata* (strain Ds2) and *P. minimum* (strain Pal1). Negative controls were inoculated with uncolonized agar plugs. For each treatment, there were six replicate cuttings. Four months after inoculation cuttings were collected. First, the length of the wood discoloration (LWD) was measured. Second, pathogens were isolated from the upper and lower margin of each wood lesion. Both the LWD caused by fungal trunk pathogens and the re-isolation rate varied considerably amongst clones. No foliar symptoms were observed during the experiment. Results showed different susceptibility levels of the vines depending on the clones and the pathogen species. In general, a higher susceptibility of vines infected by *D. seriata* was observed. Moreover, some clones showed less susceptibility to both pathogens. Therefore, this work suggests that certain clones may be promising candidates for a sustainable management of GTDs based on host resistance.

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Biological control of *Dactylonectria torresensis* with *Trichoderma* spp. in hydroponic system

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Black foot disease (BFD) is considered one of the main phytosanitary problems in nurseries and young vineyards worldwide. Biological control with *Trichoderma* species is one of the most used methods to control this disease. The aim of this study was to evaluate the effect of two *Trichoderma*-based products against *Dactylonectria torresensis*, one of the most prevalent BFD-associated fungi in Spain on grapevine. Canes of *Vitis vinifera* 'Albariño' plants were superficially disinfected with a hot water treatment (52º for 30 min) and then grown in hydroponic tanks either with or without (control) application of spore suspensions of the two *Trichoderma* products (T1 and T2). Spore suspensions of the pathogen, *D. torresensis*, were applied to the tanks for 24 h after one month growth, when most of the canes had sprouted. The canes were maintained at 20ºC with a photoperiod of 12 hours light, the water being changed weekly. One month after pathogen inoculation, basal ends of each cane were cut, surface sterilized by rinsing them in 1% sodium hypochlorite solution and washed in sterile distilled water. Small pieces of the tissues were cultured in Petri dishes with malt extract agar amended with streptomycin and incubated at 24ºC in the dark. *Dactylonectria torresensis* was isolated from 98% of the canes inoculated only with the pathogen. In treatments with Trichoderma products (T1 and T2), the recovery of D. torresensis was reduced in 68% and 91%, respectively. *Trichoderma* was re-isolated from all plants treated with *Trichoderma* products. Results of this cane assay indicate that the two *Trichoderma*-based products could be used to prevent black foot disease infections during the rooting phase in hydroponic tanks.

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Effect of hot–water treatment on Grapevine Trunk Disease fungi in *Vitis vinifera* "Albariño" plants

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Hot -water -treatment (HWT) is considered to be effective to eradicate fungal species that caused grapevine trunk disease (GTD) during the nursery process. Several studies have dealed with timing and temperatures in HWT tested in different grapevine cultivars. A protocol of 50ºC during 30 minutes is the most used in nurseries, although it could not be totally effective since 53ºC is the temperature needed to eliminate some GTD fungi, such us Botryosphaeriaceae species. The aim of this work was to evaluate the effectiveness of two HWT protocols to control GTD fungi in two year old plants of *Vitis vinifera* "Albariño" grafted on the rootstock 110 Richter. Plants, obtained from a NW Spain nursery, were all naturally infected by GTD. The experiment was carried out in a water bath with a circulation pump (Memmert WPE45) at two temperatures 50 ºC and 53ºC, for 30 minutes. Control plants were maintained during 30 minutes in a water bath but not submitted to any HWT. After treatments (HWT 50 ºC and HWT 53 ºC), plants were cut in four sections (scion, graft union, rootstock and rootstock base). The sections were peeled, surface disinfected and cultured in malt extract plates amended with streptomycin. The plates were incubated in the dark at 24ºC. At least one GTD fungal species (incluing species associated to Botryosphaeria dieback, Petri disease, Black foot disease and Diaporthe dieback) was isolated from all control plants. The treatment with water at 50º C and 53º C did not control fungi species implicated in Botryosphaeria dieback, Diaporthe dieback and Petri disease. Fungal species causing Black foot disease were not detected in both HWT treatments in *V. vinifera* "Albariño" plants.

Commercial nursery evaluation of *Trichoderma atroviride* strain I-1237 (Tri-wall®) against Grapevine trunk diseases' fungi

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Grapevine trunk diseases are some of the most widespread diseases infecting grapevine propagation material. Field trials were conducted, during two years in a commercial nursery in Bombarral, Estremadura region, Portugal, where the majority of Portugal's grapevine nurseries are located. The objective of the experiment was to assess the effectiveness of *Trichoderma atroviride* strain I-1237 (Tri-Wall®) under commercial nursery conditions (natural infection) for the protection of cuttings against Botryosphaeria dieback, Phomopsis dieback, Petri disease and Black foot disease. The effectiveness of the treatments was evaluated through the ability of this particular strain of *T. atroviride* in reducing natural infections by the fungi in propagating material. The trial was conducted successfully under local environment conditions and according to the protocol established, where *T. atroviride* strain I-1237 showed a good control of severity of diseases, despite de duration of the treatment infection strategy (1h *versus* 24h). Results obtained were similar to those obtained for cyprodinil+ fludioxonil (Switch®) which was used as standard control. *T. atroviride* strain I-1237 colonization of the cuttings was high. In conclusion, Tri-wall® can be regarded as a good and efficient biological control agent for protection of grapevine propagation materials against grapevine trunk diseases.

Interaction of *Aureobasidium pullulans* with *Phaeomoniella chlamydospora* a causing agent of Esca disease of grapevine

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Esca is a devastating fungal infection of grapevines, which primarily affect the woody tissues, but the effector molecules of the associated fungi also damage the leaves of the host. The control of the Esca-related fungi *Phaeomoniella chlamydospora* (Pch) and *Togninia minima* is still an unsolved problem partly because of the complex nature of the disease. Here we present our preliminary results about the possible positive role of a nonpathogenic-pathogenic fungal interaction in the development of the foliar symptoms of Esca disease.

In 2017, Esca-affected grapevines were monitored. The severity of foliar symptoms was measured and fungal strains were isolated from trunks. Isolates were identified by molecular methods. Trunks which contained the dimorphic fungus *Aureobasidium pullulans* (Apu) showed significantly higher symptom severity than any other group of trunks. Confrontation tests were carried out with Apu and Pch strains. As a result, both Apu and Pch strains showed decreased growth rate towards the confrontation zone, but without the death of the cells. Both Apu and Pch showed decreased growth rate in the presence of the culture filtrates of the other fungus, however the germination rates were unaffected. The production of conidia by Pch and the formation of conidia and yeast cells by Apu strains were significantly increased in the presence of the culture filtrates of the other fungal species. These effects may lead to the increased spreading of both fungi inside the trunks. Moreover, the yeast form of Apu is a potent producer of pullulan, which is a known effector molecule of Esca-associated pathogens. All these above effects possibly cause the increased disease severity on the co-infected grapevines. Our results may be explained by the presence of interspecies quorum-sensing between Pch and Apu, which may promote development of Esca disease.

The present work was supported by GINOP-2.3.2-15-2016-00061 and TÉT_16_FR projects.

Recovery and characterization of the historical varieties of *Vitis vinifera* in Siena surroundings (Tuscany, Italy)

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The genetic diversity in the genus *Vitis* to this day is not full known yet. It can be assumed that a lot of varieties of the *vinifera* species have not been fully characterized. The products that comes from *V. vinifera*, such as table grape and wine have an increasingly important role to play in the world culture, since ca. 6000-5800 BC during the early Neolothic Period.

The objective of this work is the recovery and historical landscape and environmental redevelopment of Siena through the preservation of the existent *V. vinifera* antique varieties, facing current and future challenges, preserving the biodiversity, landscape and protecting the value that these could have for the tourism in this region.

We have been able to reevaluate ignored and old native grapevines survived until now.

Through ampelographic and genetic analysis it has been possible to recover more than 40 accessions from 10 from different sites within the medieval walls of Siena and in the immediate surroundings of the town. Some grapevines have been identified, but only 20 have classified as rare and deposited in a germoplasm bank.

The deployment of a multidisciplinary historical approach, through ampelography, DNA-based marker can be helpful to preserve ancient varieties, biodiversity and can help to recover new unknown varieties. It also can be a not expensive approach in order to improve the tourism.

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Real-time PCR assay for the quantification of *Botrytis cinerea* in grapevine bunch trash

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Effective Botrytis bunch rot (BBR) management requires the reduction of bunch trash colonization and the sporulation potential by *Botrytis cinerea* at flowering and behind (Fedele et al., 2017). In the scope to better understand BBR epidemiology and evaluate the efficacy of early-season disease control, colonization rate of *B. cinerea* was assessed in grapevine bunch trash by means of real-time PCR (qPCR). The qPCR assay was optimized to quantify *B. cinerea* DNA in the presence of *Vitis vinifera* DNA in bunch trash. The fungal colonization rate, namely Pathogen Coefficient (PC) (Saito et al. 2013), was calculated for an amount of *B. cinerea* conidia added into bunch trash. The PC value increased with the increase of *B. cinerea* DNA in the presence of *V. vinifera* DNA. Besides, the PC in different bunch trash infection categories estimated by qPCR were compared to plating method and microscopic counts to evaluate the incidence of colonized bunch trash and the sporulation potential. The obtained results establish the qPCR assay as a potential tool to examine the colonization rate of bunch trash. Hence, the PC was determined in field samples to compare the effect of different fungicide treatments for BBR control. This work provides a new method to be used in studies on *B. cinerea* in vineyards.

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Early phytopathological diagnosis and new mitigation strategies for the control of viruses and trunk diseases in *Vitis vinifera* L.

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Viral and fungal diseases have become a problem of high impact for the wine industry. The establishment of pathogen-free program is a critical component of the management of vine diseases. Conventional detection tools for these pathogens demand a lot of labor. Recently, the ability to identify pathogens has improved through methods of analysis of RNA and / or DNA. Moreover, quantitative assays can be performed through qPCR and have been used to detect plant pathogens such as bacteria, fungi, and viruses, as well as a simultaneous detection approach to detect combinations of these pathogens.

Using these technologies, Viña Concha y Toro is working to establish the viral and fungal status of its main vineyards and in this way establish the bases of a quality control system for their plant production.

We are working on the implementation of a panel of 15 viruses and 6 trunk diseases associated fungi. To date, a 15% of the surfaces compromised has been analyzed for the 13 viruses and 4 trunk disease fungi considered in the project. Currently, 868 plants diagnosed as “clean” has been selected in the mother blocks to produce clean material during the 2018-2019 season. These are preliminary results but with the greatest relevance to advance in the implementation of a clean plant production system that increase the longevity of the vineyards.

Acknowledgements:

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Grapevine rootstock genotype interact with the environment to determine the rhizosphere microbiome composition

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The soil microbiome has a great interest for its potential in improving plant nutrient utilization and suppressing soil-borne diseases. This study aimed to unravel the effects of rootstock genotype on microbial communities in the rhizosphere of grapevine. For this purpose, the rhizosphere of five grapevine rootstocks (110R, 1103P, 140Ru, 41B and 16149C) was sampled at two sampling moments (June and November) in two Spanish vineyards located in Olite, Navarra (7-year-old) and Aldeanueva del Ebro, La Rioja (25-year-old) over two years. The composition of bacterial and fungal communities was examined using high-throughput amplicon sequencing of 16S rDNA gene and ITS region. Quantitative PCR approach was used to measure the relative abundance and dynamic changes of fungal pathogens associated with black-foot disease. Host genetic control of the microbiome was evident in the rhizosphere of the mature vineyard. Microbiome composition also shifted as year of sampling, and fungal diversity varied with sampling moments. Linear discriminant analysis identified specific bacterial (i.e., *Bacillus*) and fungal (i.e., *Glomus*) taxa associated with grapevine rootstocks. Host genotype did not predict any summary metrics of rhizosphere a and b diversities in the young vineyard. Regarding black-foot associated pathogens, a significant correlation between sequencing and quantitative PCR was observed. Results proved to be significantly influenced by rootstock genotype, year of sampling and vineyard location. The findings of this study provide evidence that rootstock genotype affects the process of microbial recruitment in the rhizosphere of grapevine, and this could be related to the plant age or the habitat (soil type and climate).

Improving water status and ripening in vineyards with trunk diseases: effects of a novel product based on micro–scaled technology.

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In most vineyards worldwide, agents of grapevine trunk diseases (GTD) represent a real threat for viticulture and are responsible for significant economic loss where grapevines are growing. The aim of this study was to evaluate the effects of a micro-scaled product, patented with the number PCT/ES2016/070815, on several commercial vineyards. This product has a mean size of 1 μm and it is obtained using microbial anaerobic digestion procedures. This 2-year trial (2016–2017) has been conducted in 7 *Vitis vinifera* varieties (Tempranillo, Macabeo, Syrah, Cabernet Sauvignon, Airen, Verdejo, and Garnacha tintorera). Vineyards are located at 5 viticultural areas in Spain (La Mancha, Manchuela, Rueda, Somontano, and Almansa), involving a total of 30 vineyards widely affected by GTD. Because GTD affect directly on plant water status, its measurement can yield valuable data indicative of a protective effect against the problems caused by these pathogens. Scholander pressure chamber was used for monitoring midday stem water potential in treated vs. control areas, according to standard protocols. The results showed that this micro-scaled product has significant efficacy improving water potential compared to non-treated grapevines, independently of variety and location. In grapevines treated with this novel technology, we observed a mean reduction in water stress of 18% (stemΨ variation around 0.16 MPa), compared with those non-treated grapevines. Moreover, this treatment decreased significantly the breakdown of malic acid in berries (differences around 0.68 g/L). Thus, our results showed the capacity of this micro-scaled product to improve both water status and ripening in vineyards, suggesting its ability to control GTD by direct and/or indirect mechanisms.

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Poster Session A

VALOVITIS, puesta en valor de viníferas minoritarias o en peligro de extinción en el Piedemonte pirenaico

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El proyecto Interreg POCTEFA VALOVITIS, cofinanciado por el Fondo Europeo de Desarrollo Regional (FEDER), tiene como objetivo favorecer el desarrollo de variedades innovadoras de la zona del Piedemonte pirenaico que permitan a las bodegas locales diferenciarse y mejorar su competitividad en los mercados, especialmente los de exportación.

Las variedades minoritarias prospectadas, a partir de un trabajo previo de identificación del patrimonio varietal histórico aragonés, se conservan en bancos de germoplasma. Los estudios de caracterización molecular, agronómica y enológica de estas variedades realizados por los socios del proyecto (Instituto Francés de la Viña y el Vino, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Laboratorio de Análisis del Aroma y Enología de la Universidad de Zaragoza, Centro de Transferencia Agroalimentaria del gobierno de Aragón y Estación Experimental Aula Dei - CSIC), han permitido obtener vinos de gran interés.

Dos campañas de ensayos, 2016 y 2017, en las que se han realizado más de 50 vinificaciones, han permitido evaluar el potencial de aprox. 40 variedades. En ellas se ha observado como el peso de la variedad es superior al del año, manteniéndose la tipicidad varietal de los vinos.

VALOVITIS ha puesto en valor viníferas al borde de la desaparición en el ámbito de estudio, evidenciando su escasa vulnerabilidad a plagas y enfermedades y su elevada capacidad de adaptación al cambio climático. Estas cualidades permiten paliar los efectos de este último en la calidad productiva del sector, en particular a lo relativo al aumento del grado alcohólico y a la disminución de acidez.

La identificación de viníferas con cualidades acordes a las exigencias actuales de los mercados permite que su transferencia a bodegas y viticultores pueda ser una herramienta eficaz en la mejora competitiva de la industria vitivinícola local, impulsando el dinamismo económico y social en las zonas rurales implicadas en el proyecto.

Environmental factors associated with dispersal of *Phaeomoniella chlamydospora*, causal agent of esca disease of grapevine

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Sustainable management of grapevine fungal trunk diseases (GFTDs) is difficult due to the lack of information about the biology and epidemiology of the fungal species associated with these diseases. In this work, the dispersal of aerial inoculum of *Phaeomoniella chlamydospora* was studied to improve the knowledge about its epidemiology. This pathogen, one of the most important causal agents of esca disease of grapevine, reproduces asexually by conidiophores produced in the mycelia or by pycnidia. Sampling was performed from October to May during two seasons (2015-2016 and 2016-2017) in three vineyards affected by GFTDs located in three different Spanish regions: Valencia, Logroño and Ourense. On each vineyard a weather station was installed together with 5 spore traps and rain collectors that were replaced weekly. The DNA concentration of *P. chlamydospora* was determined using a previously validated real time PCR-based protocol. Positive detections of *P. chlamydospora* were observed during all the sampling period. Twenty-six percent of the positive detections were not associated with rain events, indicating that the conidia released from conidiophores produced in the mycelia can play an important role in *P. chlamydospora* epidemiology. However, a ROC analysis indicated that rain can be a good predictor of inoculum dispersal (AUROC=0.579). Different equations were developed to describe the seasonal cumulative value of DNA detected as a function of time or degree-days: best results were obtained with a Gompertz equation function of time (R^2=0.9).

Fermentation behaviour and nitrogen requirements of *S. cerevisiae* x *S. eubayanus* and *S. cerevisiae* x *S. uvarum* hybrid strains

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From our previous study, we have observed nitrogen content in must and fermentation temperature are two important factors involved in the mixed culture fermentation between *S. cerevisiae* and cryotolerant strains of *S. eubayanus* and *S. uvarum*. *S. eubayanus* and *S. uvarum* showed higher fermentation capacity in nitrogen deficient media at low temperature. Therefore, there is a great potential to use these species in the wine industry for nitrogen-limited musts.

In order to explore the possibility to utilize the low nitrogen demanding character of cryotolerant species in wine production, we have constructed hybrids *S. cerevisiae* x *S. uvarum*, and *S. cerevisiae* x *S. eubayanus*. The nitrogen requirements and fermentation activity of the hybrids have been studied by carrying out fermentations at 4 different conditions including the combinations of high (28°C) and low (12°C) temperatures and different nitrogen abundances (60 and 300mg/L N). Additionally, we have also studied the sequential uptaking order of nitrogen sources in synthetic must by *S. uvarum*, *S. eubayanus* and the hybrids strains.

The currently results show that in general, hybrid strains complete the fermentations more efficiently than the parental strains, especially at 12°C with 60mg/L nitrogen in the synthetic must. Higher production of higher alcohols has been seen with the fermentation conducted by *S. cerevisiae* x *S. eubayanus* at low temperature. Therefore, the hybridization between *S. cerevisae*, *S. eubayanus,* and *S. uvarum* could be a good strategy to construct robust strains which are suitable for low temperature fermentation and nitrogen deficient conditions.

Microorganisms involved in unconventional alterations of wines during their conservation

Parra, A.[1]; Escribano, R.[2]; Garijo, P.[2].; López-Alfaro, I.[2]; López, R.[2]; Santamaría, P.[2]; Gutiérrez, A.R.[2]; González-Arenzana, L.[2]

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In recent years, the concern of many Spanish wineries due to the alteration suffered during the conservation of wines has increased. Generally, they are high quality wines that suffer unexpected increases in their volatile acidity during storage in the cellar. The objective of this work was to study the microorganisms involved in unconventional alterations of wines during their conservation. For this purpose, 10 wines containing high acetic acid levels (0.89-1.94 g/L), were analyzed. Following the usual control protocol in the companies, no viable populations were detected in these samples. In our laboratory, the wines were seeded in selective culture media and also analyzed by PCR (Polymerase Chain Reaction) combined with DGGE (Denaturing Gradient Gel Electrophoresis).

Only in half of the samples there were found microorganisms able to grow in the culture media used for the detection of lactic acid bacteria and yeasts, but not in the media used for acetic and environmental bacteria. Seventeen species were identified after the isolation and subsequent sequencing of 67 colonies. The analysis with PCR-DGGE allowed the identification of 15 different species in wines where viable populations had not been detected. Among the genera of microorganisms found, 36 % were lactic bacteria, 28 % environmental bacteria, 23% acetic bacteria and 13 % yeast. Altogether, 18 genera and 29 microbial species were identified, whose distribution was: 4, 7 and 15 species of acetic, lactic and environmental bacteria, and 5 of yeasts.

It can be concluded that many of the microorganisms responsible for the alteration of wines were in non-cultivable stages, and that most of the detected genera were environmental bacteria, what increases the number of species usually found during the conservation of wines.

This study was supported by the Gobierno de La Rioja Project R-09-17 and the European Regional Development Operational Program.

Control of *Brettanomyces* and acetic acid bacteria in wine with a kaolin silver complex

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Wine quality is influenced by the microorganisms that occur through winemaking. Among these, the yeast species *Brettanomyces* (*B.*) *bruxellensis* and some acetic acid bacteria (AAB) can play a negative role because of the production of undesirable off-flavor compounds. SO_2 addition has been the most effective way to reduce spoilages although nowadays there is a growing interest in the development of alternatives to its use. This work was carried out with the objective of studying the effects of kaolin silver complex (KAgC), at a dose of 1g/L in permeable bags, on controlling populations of *B. bruxellensis* and AAB. For this, two experimental essays were conducted. In Trial 1, wines were inoculated with 10^2, 10^4 and 10^6 CFU/mL of *B. bruxellensis* and AAB cultures. In Trial 2, wine was naturally contaminated with populations of 10^4 CFU/mL *B. bruxellensis* and 10^5 CFU/mL AAB, respectively. In this latter essay, KAgC treatment was compared with chitosan. Results showed that wines artificially contaminated with an initial population of 10^4 CFU/mL of *B. bruxellensis*, a concentration proven to produce off flavors in wine, contained residual populations of the contaminating yeast after 24 days of contact with KAgC. Populations of AAB inoculated in wine at concentrations of 10^2 and 10^4 CFU/mL were also reduced. In the case of 10^6 UFC/mLpopulations of *B. bruxellensis* and AAB, the effect was less marked. The antimicrobial effect of this product was also demonstrated in the naturally contaminated wine in which the populations of *Brettanomyces* and AAB decreased in a similar way that with the chitosan treatment. Moreover, silver concentration in finished wines was below the legal limits. Thus, KAgC provided effective control aganist the development of *Brettanomyces* and AAB, although it would be necessary to check if higher doses of silver complex would be even more effective in highly contaminated wines.

Interaction between yeasts in mixed non-*Saccharomyces* inocula

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The design of starters, including selected non-*Saccharomyces* yeasts with optimized biotechnological characteristics, has become one of the main challenges for researchers and oenologists, mainly for the production of wines with particular flavour profiles. With the aim of simulating complex yeast microbiota present in spontaneous fermentations, mixed cultures composed of more than one non-*Saccharomyces* species in combination with *S. cerevisiae* have also been used. In this study, the sequential inoculation approach was used to test *Lachancea (L.) thermotolerans* and *Torulaspora (T.) delbrueckii* yeasts previously selected and two co-inoculums made up of those yeasts in percentages 70% and 30%. The yeasts were inoculated at the beginning of fermentation and, three days later, a subsequent inoculation of *S. cerevisiae* was conducted. Alcoholic fermentations were performed in duplicate in microscale fermenters containing 2.5 Kg of unsterilized and non-sulphited red Tempranillo must and pomaces at 25 ºC.

Both *T. delbrueckii* and *L. thermotolerans* remained long time in tanks, participating in a great extent in fermentation. In the case of mixed cultures, both species maintained a similar percentage as when initially seeded (70/30) until *S. cerevisiae* was added, and after that they slowly decreased until they disappeared completely in seven days.

The physicochemical data of wines made with a combination of *T. delbrueckii/L. thermotolerans* showed that they were more similar to those made with *T. delbrueckii* than with *L. thermotolerans*, and it could be due to the higher quantity of *T. delbrueckii* in the inoculum (70%) and also to its higher presence during fermentation. The aroma data indicated that when those strains worked together, they contributed in a different way to aroma than when they acted separately. These differences might be caused by the interaction established between them.

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Combined effect of heat shock and must nutrition that lead stuck and sluggish wine fermentation

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Stuck and sluggish fermentations are one of the main problems affecting the wine production leading in many cases to important economic losses. Several chemical and physical factors have been associated with problematic fermentations, being exposure to extreme temperatures among them. During early fermentation stages, high metabolic activity of yeast leads to an increase in must temperature. Moreover, the nutrition of the musts with nitrogenous sources carried out during the first days of fermentation could also favour this temperature increase. The aim of this study was to identify thermal conditions leading to problematic fermentations evaluating the combined effect of heat shock and nutrition of musts. Three *S. cerevisiae* strains were used in this study. Fermentations were conducted at 28°C using synthetic must. The impact of heat shock at 36ºC and 40ºC was assessed increasing must temperature during 16 hours on the third day of fermentation. Additionally, two nutritional conditions: with and without diammonium phosphate additions were evaluated. Fermentation kinetic was monitored through density measurements whereas cell viability/vitality was evaluated with flow cytometry associated with PI and CFDA staining. Result showed that heat shock affected fermentation kinetics with different intensity depending on the temperature, nutritional conditions and yeast strain evaluated. None of the conditions evaluated leaded to a complete stuck fermentation, although sluggish fermentation was recorded. Thermal shock of 40°C lead to a delayed fermentation that triplicate the fermentation time respect to the control treatment. A high reduction in the percentages of viability and vitality of the yeasts was recorded after thermal shock. Yeast cells from fermentations with nutrition showed a higher sensitivity to heat shock; however, they showed a greater recovery, ending the fermentation before the treatments without nutrition.

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Mobile applications to prevent microbial contaminations on wines and concentrated grape juices

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The wine industry is an important economic activity for Argentina, being wines and concentrated grape juices the most important ones. Both products could be spoiled by yeasts. *Dekkera bruxellensis* is associated with phenolic flavours that have a negative impact on the organoleptic characteristics of wines, whereas *Zygosaccharomyces rouxii* is the most frequent spoilage microorganism in concentrated grape juices. The prevention of contamination is the best way to avoid microbial problems and maintain the quality of the products. Two mathematical models of prediction of *D. bruxellensis* in wines and *Z. rouxii* in concentrated grape juice were developed in our Laboratory (Sturm et al. 2014; Rojo et al. 2014). The predictive models were constructed considering the variables of the food that could be measured and modified in the industry, such as pH, concentration of ethanol and SO_2 in wines; and pH and concentration of sugars in the concentrated grape juices. The predictive models developed allow knowing which combinations of these variables may be inhibitory for the growth of the spoilage yeasts and estimate the shelf-life of the product under the storage (isothermal) and shipping (non-isothermal) temperature conditions. In an effort to provide useful and practical tools with easily access to winemakers and producers, mobile applications were developed. The use of these Apps will contribute to the reduction of defects and alterations in the wines and juices of concentrated grapes avoiding rejections in exported items resulting in a reduction of economic losses associated with these problems.

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Winemaking by-products as natural tools in the control of the foodborne pathogen *Campylobacter*

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Grapes are one of the world's most commonly produced fruit crops. Grapes and winery industries produce a great variety of wines, grape juices, and raisins, but its production process generates high amounts of by-products, such as grape pomace, seeds, skins, stems, leaves and lees. These winemaking by-products contain numerous bioactive compounds, such as dietary fibre and phenolic compounds, with potentially bioactive activities against foodborne pathogens. Among them, *Campylobacter* is the leading cause of bacterial foodborne gastroenteritis worldwide and in the last years, the rise in the incidence of infections caused by antibiotic-resistant strains makes this illness increasingly difficult to treat. Accordingly, the tendency for searching natural and sustainable strategies to reduce the incidence of *Campylobacte*r is rising.

In the present work, several winemaking by-products were tested against different strains of *Campylobacter* (*C. jejuni* and *C. coli*). The results obtained showed that most of them had antimicrobial activity against *Campylobacter*, but grape seed extract (GSE) proved to be the most effective as antimicrobial. As minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of the GSE were in the range between 10 and 100 mg/L, GSE extract could be considered as a promising antibacterial agent potentially useful for the control of *Campylobacter* in foods. In addition, infective isolates coming from campylobacteriosis patients rendered higher levels of proinflammatory cytokines (IL-6, IL-8, MCP-1, and TNF-α). Treatment of human intestinal epithelial cells with GSE significantly reduced cytokines production in a dose-dependent manner, contributing to improve disease prevention or reduce disease severity. Finally, the fractioning and phenolic characterization of the active extracts showed that catechins and proanthocyanidins were the main compounds involved in the antibacterial effect.

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Optimization of culture conditions for the production of β–glucosidase from an uruguayan strain of *Issatchenkia terricola* and study of aroma release in C. Sauvignon wine.

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A major fraction of aromatic compounds are linked to sugars, as non-volatile compounds, representing a significant reservoir of aroma to be released (González-Pombo et al., 2014). β-D-glucosidase is a key enzyme in the enzymatic hydrolysis of these aroma precursors and thus, an useful tool to be use in the development of aroma. β-glucosidase from *Issatchenkia terricola* is a monomer of 48 kDa with an isoelectric point of 3.5. It is tolerant to glucose and ethanol, properties compatible with enological use. It has been demonstrated to be active against several aroma precursors of muscat wine, showing more specificity towards norisoprenoids (González-Pombo et al., 2011). As these compounds are associated to honey, nuts and raisins notes, it is interesting to study this enzyme in a red wine. A limitation in it use is the usual low productivity when wild strains are used. In this work, culture conditions were modified in order to improve enzyme production as well as the aroma profile of Cabernet Sauvignon was studied after wine enzymatic treatment. Enzyme productivity was increased 2-fold in a synthetic medium with glucose as carbon source with respect to the YPG (control medium) and 3 to 8-fold with respect to other media assayed. Enzyme production increased concomitantly with O.D., showing that it is associated with yeast growth. The maximum production of enzyme was observed at 28° C and pH 5-6. The scaling up was assayed in both, batch and fed-batch culture. Although the latter is usually the most suitable system for enzyme production in submerged culture, in this case the yield was practically the same as in batch culture.

GC-MS analysis of aglycones after enzymatic hydrolysis revealed significant increases in the concentration of phenols (83%) and norisoprenoids (65%) resulting in a wine with dried fruits and raisins notes (de Ovalle et al., 2018).

Phenolic composition of monovarietal red wines: volatile phenols and precursor compounds

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Volatile phenols in wines are responsible for unpleasant aromas which affect the wine quality. These compounds are produced from the microbial degradation of hydroxycinnamic acids (Shinohara et al 2000; Wendral et al 2010;). The aim of this study was to characterise wines from different grape varieties focusing on volatile phenols and on the respective precursor compounds. Fifty-eight commercial monovarietal red wines from eight selected grape varieties were used: Cabernet Sauvignon, Syrah, Aragonez, Castelão, Touriga Franca, Touriga Nacional, Trincadeira and Vinhão. The volatile phenols precursors, both on the free form (p-coumaric, ferulic and caffeic acids) and as tartaric esters of hydroxycinnamic acids (caftaric, coutaric and fertaric acids), were measured by HPLC-DAD, LC-MS. The volatile phenols were analysed by GC-FID. It was found that volatile phenols precursors exist mostly as esters of tartaric acid, with caftaric acid as the most abundant cinnamate, followed by coutaric and fertaric acids. The predominant hydroxycinnamic acid in the free form was p-coumaric acid. Touriga Nacional exhibits the highest difference between bound and free forms. Twenty two % of the wines analysed presented levels of volatile phenols above the perception threshold, with ethylphenols clearly predominating over vinylphenols. These volatiles were highest in Vinhão and Trincadeira, showing an average value well above the perception threshold. The concentrations found in Cabernet Sauvignon and Syrah wines were around 10 times lower than those reported in previous works.

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Enological Potential of *Hanseniaspora vineae*

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During alcoholic fermentation, yeasts transform sugars present in the grape must primarily into ethanol, glycerol, and carbon dioxide, as well as other metabolites that contribute to the final quality of a wine. Because these metabolites are important for the sensory quality of wines, it is important to understand that winemaking is favored by increased yeast diversity. This strategy is the ideal way to create unique wine profiles within a global market. The aim of this work is to study the oenological potential of some strains of *Hanseniaspora vineae*, isolated from Uruguay.

Semi-industrial fermentations of Macabeo must were inoculated with *H. vineae* or *S. cerevisiae*. The presence of *H. vineae* throughout the fermentation resulted in more fruity and flowery wine, as indicated by the chemical analysis of the final wines, which demonstrated a strong presence of 2-phenethyl acetate at concentrations higher than the threshold of perception and approximately 50 times more than that produced in wines fermented with *S. cerevisiae*. This compound is associated with fruity, floral and honey aromas.

Other fermentations were performed with different *H. vineae* strains in model grape juice medium to determine the sensory properties related to consumer preferences. The results obtained enabled the verification of variability between strains of this species for some key attributes. The accumulation of some key aroma compounds was also consistent, including low production of medium chain fatty acids, ethyl esters and tyrosol, and high production of acetate esters and sesquiterpenes compared to *S. cerevisiae* strains. The most outstanding characteristic of *H. vineae* was the higher production of flavor compounds derived from the aromatic amino acids phenylalanine and tyrosine, such as benzenoids and phenylpropanoid esters. The presence and high expression of *ARO* genes as a result of duplications in *H. vineae* genome might explain these metabolic differences with *S. cerevisiae*.

Enological and viticultural practices impacting *Saccharomyces cerevisiae* strains and yeast species biodiversity in vineyards

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The main objective of this study was to compare different viticulture and enological practices to determine their influence on the biodiversity of *Saccharomyces cerevisiae* wild strains. The study was conducted at *S. cerevisiae* strain level, 1016 *S. cerevisiae* isolates were obtained from four vineyards, genotyped by interdelta analysis and differenced by using a custom computer software developed in this work. Ancient vineyards managed with organic practices showed intermediate to low levels of *S. cerevisiae* biodiversity whereas conventional practices increased the number of strains detected. In this study, the use of autochthonous vs allochthonous *S. cerevisiae* strains to conduct fermentations seemed to be especially determinant. The study showed a drastic reduction on *S. cerevisiae* strains diversity in a winery using an autochthonous-selected *S. cerevisiae* strain for wine inoculation, being the only one detected in vineyards, probably due to the bio-increment of a strain that could be highly adapted to the vineyard from where it was originally isolated. The use of commercial yeast strains is a common practice in winemaking, avoiding stuck or sluggish fermentations and, leading to a predictable quality in wine production. However, in line with the conclusions of this work, the use of commercial yeasts can lead to a consequent reduction of autochthonous yeast biodiversity.

Phenotypical characterization of *Saccharomyces cerevisiae* wine strains with different *IRC7* genotypes: implications on growth ability.

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Varietal thiols contribute to the characteristic aroma of white wines. Yeasts uptake odorless precursors of musts and latter cleavage them by β-lyase enzymes (mainly Irc7p), releasing the volatile thiols.

IRC7 gene has two isoforms; a complete version and a deleted form, being the β-lyase of the latter less active on thiol production. In this study, the *IRC7* genotype of 252 *S. cerevisiae* wine strains were determined, finding a greatly unbalanced situation on the population distribution: the homozygous complete version of *IRC7* was present in only the 4% of the strains.

It has been described that the complete *IRC7* allele is related to an introgressed segment (derived from *S. paradoxus*) near the telomere of chromosome VI. In addition, i*n silico* analysis revealed that relevant genes such as *HXK1*, *RET2* or *RPN12* are closely located to *IRC7*.

To explain the markedly lower presence of homozygous for the complete allele we performed a HTS phenotyping study to determine if the presence of this allele implies any metabolic disadvantage. Thirty strains (10 strains representing each *IRC7* genotype; homozygous for the short allele, heterozygous and homozygous for the complete allele) were microcultivated in a panel of 50 media (testing different carbon and nitrogen sources, physic-chemical conditions and toxins). Lag time, growth rate and efficiency were obtained from cultures, and compared between each genotype group.

This study indicated that homozygous strains for the complete allele (therefore, with higher potential on thiol releasing) establish an aside group, showing mostly higher lag times and lower growth rates in most of the conditions tested. Since the metabolic function of this gene is not as important to explain the marked *IRC7*-associated growth ability, all these data suggest that genome scale recombination may occurred, implying both *IRC7* related regions and other genome regions.

Impact of mepanipyrim and tetraconazole commercial formulations on the proteomic expression of Lalvin T73™ in synthetic must

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This work is a proteomic approach of the impact of some commercial formulations on the growth of *Saccharomyces cerevisiae* var. *bayanus* Lalvin T73™ when a nitrogen restrictive medium is used.

Alcoholic fermentation assays were performed under semi-aerobic conditions with a synthetic must using only phenylalanine as nitrogen source (160 mg N/L, n=6). Bottles was supplemented, separately, with two commercial formulations: 12.5 % (w/v) of tetraconazole and 50 % (w/v) of mepanipyrim at a concentration levels corresponding to two times their maximum residue limits in grapes according with the European legislation (Regulation (EC) Nº 396/2005 and later modifications). A control experiment (without fungicides) was also performed for comparative purposes. At regular intervals along fermentation (10 days), aliquot samples were taken and yeast cells were removed from media by centrifugation.

Soluble protein extracts were obtained following the method proposed by Von der Haar [1] with modifications. So, 50 mg of yeast sample was treated with a lysis buffer containing 0.1 M NaOH, 0.05 M EDTA, 2 % β-mercaptoethanol and a protease inhibitor. Proteins were digested with trypsin and analysed by high performance liquid chromatography tandem mass spectrometry. After protein identification using the MASCOT search engine, Venny diagrams comparing each fungicide experiment respect to the control were done, and proteins classified according their biological process.

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Can iprovalicarb, mepanipyrim and tetraconazole modify the proteomic profile of Lalvin T73™ yeasts?

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Stress situations, such as the presence of fungicide residues in must, may induce changes on the proteomic expression of wine yeasts, modifying the yeasts metabolic pathways and the sensory characteristics of wines. The aim of this work was to evaluate the effect of fungicide residues on the activity of *Saccharomyces cerevisiae* var. *bayanus* Lalvin T73™.

Vinification experiments were performed, by triplicate, with *Vitis vinifera* var. *Monastrell* grapes. Grapes (8 kg per fermentation) were crushed and the must was, separately, supplemented with mepanipyrim, tetraconazole and iprovalicarb standard solutions at two concentrations levels corresponding to two and five times their Maximum Residue Levels (MRL) on grapes (Regulation (EC) Nº 396/2005 and later modifications). Control wines (without fungicides) were also obtained for comparative purposes. A total of 21 vinifications were carried out.

At the end of fermentation, yeast cells were removed by centrifugation and submitted to a protein extraction procedure based on the method proposed by Von der Haar [1]. Yeast cells were resuspended in lysis buffer (0.1 M NaOH, 0.05 M EDTA, 2 % β-mercaptoethanol and a protease inhibitor) and immediately heated to 90 ºC, thus achieving simultaneous lysis and solubilisation. Then, extracts were submitted to shotgun protein analysis by HPLC-MS/MS. After protein identification, Venny diagrams were done in order to compare each fungicide experiment with the control sample. Not common proteins for each Venny diagram were classified according to their biological process to know which kind of proteins showed the greatest modifications.

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[1] Von der Haar, T. PlosOne 2007, 2, e1078.

Reconstruction of *H. guilliermondii* UTAD222 metabolic network reveals new potential genes involved in acetate ester formation

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It is known that *Hanseniaspora guilliermondii* affects the production of volatile compounds of wines when in consortium with *Saccharomyces cerevisiae*. Consistently, it has been reported the ability of *H. guilliermondii* itself to produce compounds having an impact in wine aroma even in single culture, in particular ethyl acetate. To better understand the role played by *H. guilliermondii* in the context of wine fermentation and in particular in aroma modulation, we have recently performed an extensive functional analysis of the *H. guilliermondii* UTAD222 genome. We were able to reconstruct the metabolic network of this strain having identified a set of genes predicted to be involved in aroma formation, this representing the *H. guilliermondii* "flavorome". Notably, within this cohort of proteins we could not identify homologues for known acetyl transferases, involved in formation of ethyl acetate, contrasting with the reported high production of this molecule by *H. guilliermondii*. A closer analysis of the *H. guilliermondii* UTAD222 ORFeome led us to identify four proteins that harbor motifs conserved within the alcohol acetyltransferase enzyme family, these proteins only having orthologues in other species of the *Hanseniaspora* genus. Functional analysis of these genes is being performed and their role in formation of aroma compounds in single and mixed-culture fermentations of *H. guilliermondii* with *S. cerevisae* examined.

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The WM&B_Lab yeast culture collection: biodiversity of non-*Saccharomyces* as a source for the development of new starter-cultures.

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Preserving yeast diversity is becoming particularly important, as the use of yeast species other than *S. cerevisiae* for industrial applications is increasing. In particular, there is an increasing interest in non-*Saccharomyces* yeast strains which occur naturally in grape must as they have proved to be highly promising in improving or at least diversifying the chemical profile of wines. Herein we report a high-throughput phenotypic screening of a large collection of non-conventional yeast species isolated from the winemaking environment for desirable stress tolerance and other technological traits of interest for winemaking production. We have found that within our yeast collection there is a broad diversity of species and strains within a species that could be useful for exploitation not only in winemaking but also for other biotechnological applications.

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Evaluation of the fermentative proprieties of an autochthonous *Saccharomycodes ludwigii* strain for application on alcoholic beverage industries

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The fermentative potential of a non-conventional yeast of *Saccharomycodes ludwigii*, tradicionally described as a wine spoilage yeast, was evaluated to explore its potential use for alcoholic beverage production. As such, fermentations of a natural grape-juice have been performed either in single or in mixed-culture with the commercial *S. cerevisiae* QA23 strain, inoculated at the beginning or 72h after *S. ludwigii*. The results show that this non-*Saccharomyces* yeast strain was able to ferment all the sugars present in a reasonable period of time without impairing the proprieties of final wine as compared with *S. cerevisiae* single fermented wines. On the other hand, when in mixed culture with *S. cerevisiae*, a slight but yet significant decrease in ethanol levels was detected. Furthermore, its presence enhance the production of esters compounds associated with the fruity and floral aroma, in particular ethyl octanoate and 2-phenyl-ethyl-acetate. Based on our resuls, it can be foreseen a possible use for this indigenous non-*Saccharomyces* yeast either in monoculture fermentations or as co-starter culture with *S. cerevisiae* for the production of high quality alcoholic beverages. More studies should be conducted particularly examining the sensory qualities of the final products obtained.

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Evaluation of oenological proprieties of natural *Saccharomyces cerevisiae* for production of Douro Region wines

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The systematic use of *S. cerevisiae* commercial-starters in wine industry has led to the masking of the distinctive peculiarities of wines produced in different geographical regions. Thus, the exploitation of the microbial diversity that exists in the vineyards has been considered an interesting approach to overcome this concern. It is assumed that autochthonous *S. cerevisiae* populations are more well adapted to local conditions and that differentially affect the chemical and sensory profiles of wines underlining its *terroir*.

In this line, a set of natural *S. cerevisiae* isolated from Douro Region wine have been surveyed for genomic diversity and for relevant oenological proprieties, such as resistance to stress, as well as their ability to adjust to different grape-must environments and potential to produce aroma compounds. Some natural *S. cerevisiae* strains outcompeted the commercial strains for specific traits. Herein, data resulting from several fermentations with selected natural strains and grape-juices will be presented and discussed, together the chemical profiles of the wines obtained. The envisioned use of these strains it is of relevance for the Douro Wine Region industry as a mean for product valorisation, as wine tipicity is increasingly seen as a quality indicator for consumers.

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Genome-wide phenotypic and transcriptomic profiling unveils an essential role of the Com2-regulatory system in response and tolerance of *Saccharomyces cerevisiae* to sulphur dioxide (SO_2)

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Sulphur dioxide (SO_2) and its derivatives are widely used as preservatives in the food and beverage industries which rely on its antimicrobial properties to assure microbiological quality of their products. The emergence of highly SO_2-tolerant spoilage strains from different species have led to an increase in the concentrations used in the food industry, a practice that has human health adverse effects besides exerting selective pressure for more tolerant strains. In this study, we took advantage of the central role of *S. cerevisiae* (itself a spoilage yeast) as an experimental system to shed light into the alterations occurring in yeast genomic expression during early response to SO_2 and the involvement of the transcription factor Com2 in this transcriptional reprogramming. Com2p was found to regulate directly or indirectly, the transcription of 80% of the SO_2-activated genes. Large-scale phenotypic screening of an haploid deletion mutant collection have led to the identification of 767 determinants of yeast resistance to SO_2, 47 of these being identified as Com2-targets. The role of the Com2-regulatory system in the extreme resistance to SO_2 registered in strains recovered from wine must will also be discussed. On the overall, the results emerging from this study provided insights into the molecular response of yeast cells to SO_2 in *S. cerevisiae*, specially in wine strains, a knowledge that can be used to develop of more suitable and safer preservation strategies in the food industry.

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Yeast biogeography in organic and conventional grapes and musts from Galicia (NW Spain)

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The influence of organic versus conventional farming system on biogeographical patterns of yeast communities was evaluated in grapes and musts from four Denominations of Origin (DOs) from Galicia (NW Spain). 42 grapes samples were collected and yeast diversity was characterized at the species level. Predominant species, *Aureobasidium* spp., *Metschnikowia* spp., *Hanseniaspora uvarum* and *Cryptococcus* spp. were widely distributed; however, their proportion differed depending on cultivation system, variety, sample (grapes or must) and location. In addition, important differences were found in the minority species, which were identified mainly in organic farming. ANOSIM and SIMPER analyses evidenced some qualitative differences in yeast communities between DO. A biogeographic pattern in the yeast species distribution was associated with each region; *Issatchenkia terricola, Starmerella bacillaris, Zygoascus hellenicus/meyerae* and different species of *Candida, Pichia* and *Zygosaccharomyces* genera were isolated almost exclusively in Ribeira Sacra and Rías Baixas DOs, whereas *Lachancea thermotolerans* was ligated to Monterrei and Ribeiro DOs. At statistic level PCA and climatic CCA confirm these differences. Our findings corroborate the existence of a microbial *terroir*, especially in organic farming where yeast diversity was higher. Therefore, organic production in Galician vineyard contributes to maintenance of wine typicality and regional diversity of yeasts with potential use even in fields beyond the oenological domain.

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The selection of low-yield ethanol evolved *Saccharomyces cerevisae* and non-*Saccharomyces* strains

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There has been a collective effort by wine researchers in the last decades to develop strategies to lower ethanol content of wines. The use of non-*Saccharomyces* yeast, in mixed inoculations with *Saccharomyces cerevisiae* strains, has been a successful approach, although with some limitations, such as the oxygen requirement or the high acetate production. This research aimed to obtain low-yield ethanol *S. cerevisiae* and non-*Saccharomyces* strains with optimal fermentative characteristics. In a previous study, non-GMO approaches, such as adaptive evolution, were used in order to obtain evolved *S. cerevisiae* strains with enhanced performance to specific wine fermentation environments. Nutritional environments were tailored to impose several selective pressures for which evolutionary enhancements are of industrial relevance (high sugar, high ethanol, nutrient limitation, etc.). The fermentative performance and ethanol yield production has been analyzed on those evolutionary enhanced wine yeasts. In terms of non-*Saccharomyces* yeasts, 51 strains belonging to 18 different species were also screened in synthetic must fermentation in laboratory conditions. During synthetic must fermentation, density, cell population, sugar consumption, ethanol production and genetic characteristics were analyzed. According to the current results, several strains with low ethanol production were selected because of their reduction on the ethanol yields up to 50 %, compared to *S. cerevisiae*. The strains that showed significant reductions belonged to the species *Metschnikowia pulcherrima*, *Issatchenkia terricola*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii* and *Hanseniaspora uvarum*. Our results suggest that this combined strategy of the selected non-*Saccharomyces* strains in mixed fermentations with the evolved *S. cerevisiae* strains is a promising approach for decreasing the ethanol content in wines.

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Interactions between *S. cerevisiae* and *T. delbrueckii* yeasts during alcoholic fermentations

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The oenological interest in the use of non-*Saccharomyces* species is increasing, mostly due to their positive contribution to the final quality of the wines. These species are mainly used in mixed inoculation with *S. cerevisiae*, although the interaction between them is not completely understood. The aim of this study was to analyse the interactions between *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains during alcoholic fermentations. Different combinations of strains of both species were used to ferment natural and synthetic must at 16ºC, in sequential inoculation. Fermentations were first inoculated with *T. delbrueckii*, and when the density decreased 15 units, *S. cerevisiae* was added. In another condition, the medium was centrifuged and filtered before the inoculation of *Saccharomyces*, in order to eliminate the presence of *T. delbrueckii* from the medium. Single fermentations of both species were used as a control. The fermentation kinetics and yeast growth and imposition was followed over time. Our results showed a delay on the mixed fermentations, compared with the single *S. cerevisiae* inoculation. Indeed, the imposition of *Saccharomyces* was impaired in sequential fermentations. Unexpectedly, the condition in which *T. delbrueckii* was removed from the medium resulted in stuck fermentations, indicating some inhibitory effect on *S. cerevisiae*.

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Influence of grape varieties and different vineyard pruning methods on grape yeast populations

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Wine quality is influenced, partially, by the composition of grape juice and the microorganisms present in the fermentation process [1]. Grapes are a primary source of natural yeasts in wine production. The population density and the diversity of indigenous yeasts on the grape berries are intrinsically linked to numerous factors; including those associated with vineyard management [2, 3]. Consequently, for winemakers it is important to have more information about the yeast communities present on grapes in order to produce high quality wines with representative characteristics, therefore a deeper knowledge of the effects of these factors on the yeast ecology of grapes is required. Hence, the aim of this work was to obtain preliminary data concerning the influence of different vineyard pruning methods and grape varieties on grape associated yeast microbiota. To this end, three methods of vineyard pruning (double guyot as control, triple guyot and smart), with three different red grape varieties used recently for red winemaking in Andalusia (Shyraz, Tempranillo and Tintilla de Rota) were selected. A total of 105 isolates from 27 samples were collected. Theses isolates were identified by rep-PCR using $(GTG)_5$ oligonucleotide [4], and analysed and clustered by Bioinformatic software. These preliminary results indicated an influence of the grape variety on grape associated yeast biodiversity while the influence of the pruning method used in the vineyard was less noteworthy.

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Analysis of the growth behaviour of three varieties of *Saccharomyces cerevisiae* in a synthetic must with different nitrogen conditions

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The viability of alcoholic fermentation depends on nitrogen source, among other factors. A low content can produce stuck or sluggish processes. In this way, yeast strains can show different preference by nitrogen source. In this work, three *Saccharomyces* strains were evaluated in the presence of several nitrogen sources at different concentration levels.

Fermentation assays were done in synthetic must at laboratory scale (400 mL), under semi-aerobial conditions. Musts were inoculated, separately, with *S. cerevisiae* var. *bayanus* (Lalvin T73™) commercial strain, *S. cerevisiae* var. *uvarum* (BMV58) and *S. cerevisiae* var. *bayanus* (EC1118), last two were isolated by reserchers of IATA-CSIC. Lalvin T73™ was tested with 3 aminoacids individually: phenylalanine, valine and leucine at 160 mg N_2/L. BMV58 and EC1118 were evaluated with phenylalanine at two concentration levels (160 and 300 mg N_2/L) and with a mixture of phenylalanine (180 mg N_2/L) and ammonium (120 mg N_2/L). Musts were incubated for 7 days with agitation, in a thermostatically controlled chamber at 21ºC. At regular intervals, liquid samples were collected to analyze yeast growth through biomass measurement (OD 650 nm) and fermentation parameters (sugars, ethanol, acetic acid and glycerol content) by HPLC-IR [1].

As expected, each yeast strain response was different depending on nitrogen origin. Lalvin T73 fermented in all tests media except with leucine. EC1118 had higher requirements of nitrogen; better results were obtained with 300 mg N_2/L, reaching the double of its growth compared with 160 mg N_2/L concentration. By contrast, BMV58 was unable to start the fermentation regardless of the nitrogen source considered.

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Cinnamoyl esterase activity in *Oenococcus oeni*

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Some strains of *Oenococcus oeni* have been found to possess cinnamoyl esterase activity that can be relevant in the malolactic stage of wine production by liberating phenolic acids, precursors of volatile phenols responsible for sensory faults in wine. The objective of this study is to better understand the basis of this differential activity between strains. Five strains of *O.oeni* were selected, three exhibiting cinnamoyl-esterase activity (CE+) and two not (CE-). Pasteurized wine was used as source of cinnamate esters in growth and metabolism experiments whilst trans-caftaric acid was used as substrate for enzyme assay. On analysis of full genome sequences previously provided for all strains, no specific gene could be found that was common only to the CE+ strains. Initial results show that, unlike the free, caffeic form, trans-caftaric acid is not toxic toward *O. oeni*. The CE activity is apparently intracellular and present in wine-exposed and unexposed strains. The protein concentration in cell free extracts of unexposed strains was more than twice that of the exposed ones. Only in the case of the CE+ strains exposed to wine did the cell debris contain higher protein concentrations that the unexposed ones. Cell free extracts of the 3 CE+ strains, totally degraded trans-caftaric acid to trans-caffeic acid, whilst extracts of CE- strains exhibited a lower activity, albeit higher for one of these strains in experiments where there had been no prior exposure. In the case of CE+ strains, wine-exposed samples showed a more rapid degradation than the unexposed ones. These results highlight the possible implication of membrane transport proteins in cinnamoyl esterase activity shown by CE+, *O. oeni* strains studied here.

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Impact of different wild yeasts on the aromatic profile of cv. Verdejo wines

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The use of wild, native or indigenous yeasts to ferment wines enhances the microbial diversity and the oenological characteristics. The selection of the appropriate indigenous yeasts is useful to increase the wine aroma complexity and to avoid fermentation risks related to spontaneous alcoholic fermentations (AF). In this study, indigenous yeasts from Verdejo grapes grown in D.O. Rueda have been identified, characterized and selected, in order to improve the aromatic complexity of wines. Yeast strains were isolated at three different stages throughout the AF. A total of 6 strains of *Saccharomyces cerevisiae* and one non-*Saccharomyces* species were identified by Delta elements and RFLP-ITS techniques, respectively. The non-*Saccharomyces* species identified was *Metschnikowia pulcherrima* which is commonly related to a high production of some aromatic compounds, such as thiols and terpenes. All isolated strains were characterized at laboratory-scale in fermentations made by pure and mixed cultures. Finally, all the identified strains were used at semi-industrial scale in order to determine the aromatic profile of the produced wines. The yeasts growth and the fermentation progress were monitored by viable cell counts and density measurement, respectively. The imposition of the inoculated yeast were also considered. At the end of the fermentations, the oenological parameters and the aromatic profile of wines were determined by GC-MS and sensory analysis. Results show that the use of different indigenous strains involved significant oenological differences. It should be noted that one pure culture of *S. cervisiae* and its mixture with *M. pulcherrima* clearly improved the aromatic profile of Verdejo wines.

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Evaluation of wild lactic acid bacteria from Rioja and Ribera del Duero Tempranillo wines

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The malolactic fermentation (MLF) process plays an integral role in the flavour and the sensory profile of red wines and also provides an important microbial stabilization. The MLF is commonly made by the lactic acid bacteria (LAB), among them *Oenococcus oeni* is the main responsible to carried out this process. However, the MLF development could be limited under some oenological factors, such as pH, sulphur dioxide and ethanol content, etc. The objective of this study was to identify and characterize the wild LAB strains and also evaluate their genetic ability to produce biogenic amines. For this, LAB strains were isolated from 10 spontaneous fermentations of Tempranillo grapes cultivated in D.O.C La Rioja and D.O. Ribera, in 2016 and 2017 vintages. Different LAB species of *Lactobacillus*, *Pediococcus* and *Oenococcus* were identified by Restriction analysis of the amplified 16S-rDNA. Furthermore, typification of *Oenococcus oeni* was done by multiplex RAPD-PCR analysis. The *O. oeni* strains without the ability to produce biogenic amines were characterized in laboratory scale fermentations. Microbiological, chemical and sensory analyses were made in order to evaluate the oenological potential of these strains. The wines produced by the selected *O. oeni* strains showed interesting organoleptic and chemical properties. These *O. oeni* strains could be use as starter culture, and help to control the biogenic amine content in wines, preventing their health related effects.

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Prions do not seem to be relevant for either sluggish fermentation or reduced alcohol yield

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Recent reports indicate [*GAR*+] prion-like elements could influence ethanol yield during wine fermentation. These prion-like elements partially relieve carbon catabolite repression, and increased respiration was one the proposed mechanisms. However, supporting data are not truly consistent (1,2). We have obtained and characterized [*GAR*+] derivatives from wine yeasts with different genetic backgrounds. The characterization included carbon catabolite repression, different factors affecting prion state appearance and stability, and the physiological impact in terms of growth and metabolic yields under aerobic and anaerobic fermentation conditions with natural grape must.

Our thorough characterization of different yeasts strains shows clearly different frequencies of prion apparition, and prion stability in desiccation experiments; highlighting the importance of the genetic background. However, concerning natural grape must fermentation, no advantage for [*GAR*+] strains was detected. For instance, in some prion strains, the [*GAR*+] phenotype is undistinguishable from that of the [gar-] version. However, for other strains, fermentations of natural grape must inoculated with [*GAR*+] yeast had a reduced fermentative performance in terms of residual sugar or CO_2 release; while the impact on ethanol content was null or almost negligible. Our results do not support a practical use of [*GAR*+] in winemaking; and rule out prions as an explanation for sluggish or stuck fermentation.

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Morphological and proteomic characterization of yeast extracellular vesicles in an oenological context

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Fungal extracellular vesicles (EVs) were first characterized about a decade ago. However, our current knowledge indicates they are ubiquitous in nature. EVs are membranous structures that are able to go across the cell wall and release different molecules to the outer cell space. The released molecules (proteins, lipids, nucleic acids, polysaccharides and pigments) have diverse functions such as cell-to-cell communication, response to cellular nutrient availability, biofilm formation, cell defense, RNA export, morphological transition, prion transmission and modulation of the host defense machinery (1–3)Springer Science+Business Media, LLC. Purpose of Review: Fungal extracellular vesicles (EVs. Despite all these important functions, EVs have not been previously studied from wine yeast species neither under winemaking conditions.

We have, for the fist time, isolated and physically characterized extracellular vesicle production of seven strains of wine yeast, two *Saccharomyces cerevisiae* and five non-*Saccharomyces* isolates, in synthetic grape must. With this purpose, we isolated the EVs from the cell culture supernatant of all yeast isolates. We report the visualization and the morphological characterization of the EVs by transmission electron microscopy and dynamic light scattering. Furthermore, we have performed a complete proteomic analysis by LC-MS/MS of the EVs from some of these wine yeast strains.

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Interaction with *M. pulcherrima* promotes a transcriptional Crabtree like effect response in *S. cerevisiae*

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In an effort to differentiate products, the wine industry is incorporating non-*Saccharomyces* yeasts as starter cultures. This allows, for the first time, more than one major species to coexist during fermentation. Our group is interested in studying the transcriptomic effects that this new trend has over *S. cerevisiae* during co-cultivation. We have shown how these non-*Saccharomyces* yeasts influence the transcriptome of *S. cerevisiae* under aerobic conditions (conditions similar to the start of fermentation process) and anaerobic conditions (as for most of the fermentation process). The strains assessed have shown how co-inoculation produces a response involving glucose uptake and glycolysis, cell wall lipid metabolism, and the use of alternative nitrogen sources. In this new work with *M. pulcherrima*, this aerobic specialist yeast triggers the strongest transcriptional effect over glycolysis that we have observed so far. We describe this transcriptional reconfiguration as "Crabtree like effect" because of the parallelism with this metabolic trait. One hypothesis that we are testing points to the higher dissolved oxygen needs of *M. pulcherrima* as a trigger for this response. This works shows how different species have different necessities during the wine making processes and they have to be taken into account to achieve the desired results.

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Interactive room with dynamic sensory stimulation technology for the enotourism diffusion activities of Arequipa region

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In the present work, an interactive room with dynamic sensory stimulation technology (DSE) that allows to show Pisco and wine organoleptic features is implemented and evaluated. The Dynamic Sensory Technology allows visitors to get a comprehensive sensory capture, consisting of: a) climate stimulation: similar to vineyards, fermentation zone, distillation room, etc., mainly in temperature and relative humidity, b) olfactory stimulation : from different environments and varieties of Pisco and wine (controlled nebulization), simulating fermentation and distillation processes, c) visual 3D stimulation: with details from planting to harvesting, including wine products elaboration, d) taste stimulation: products tasting. With the application of this technology, the interactive room will allow: a) promote wine and Pisco culture.

Sensory properties of red sparkling wines elaborated by different oenological techniques

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This study evaluated the effect of different oenological techniques on odour activity value (OAV) [1], foam [2] and sensory characteristics [3] of red sparkling wines elaborated with Tempranillo. The techniques studied were, pre-fermentative maceration with dry ice (PM-DI), delestage with partial remove of seed (PM-D), sugar reduction in must by nanofiltration (M-SR), partial dealcoholization by reverse osmosis (M-AR), carbonic maceration (PM-CM) and maceration with pectolytic enzymes (PM-E). The most powerful odorant red sparkling wine was PM-DI, followed by M-SR. PM-E and PM-CM red sparkling wines showed the lowest total OAV. Relationships between instrumental and sensory variables were analysed trough Principal Component Analysis. Regarding foam evaluation, PM-DI and M-SR red sparkling wines were correlated with HM and bubble size, and thus related with higher foamability. M-AR and PM-D red sparkling wines showed higher correlations with foam stability. PM-E was closely related with effervescence and foamability while PM-CM red sparkling wine was characterized by better perceptions of foam descriptors. Finally, in the gustatory sensory analysis, PM-DI red sparkling wine showed low levels of acidity and freshness, M-AR was correlated with astringency, and M-SR red sparkling wine was the best-valuated regarding equilibrium, persistence and mouth feel. The PM-E red sparkling wine was correlated with freshness and low bitter, and PM-CM with low persistence.

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Unravelling the basis of wine texture

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Wine intake imparts a sensory experience involving not only the wine aroma and taste but also the texture feeling or mouthfeel. At mouth, the wine interacts with the saliva layer that covers the oral cavity, stimulating the filiform papillae that contains numerous mechanoreceptors; as a result, information about wine texture is sent to the brain. Although some previous studies have evaluated the influence in wine texture of certain major wine components there is still some confusion regarding the actual role of each of these components and, especially, there is a lack of knowledge regarding how to characterize wine texture instrumentally. This work aims to investigate wine components traditionally associated to textural sensations by using rheology and density measurements.

Due to the great complexity of wine, a sensory-flat wine (white alcohol-free wine) was chosen and the following food-grade ingredients were added: glycerol, ethanol, oak tannin and mannoproteins. Samples were mixed with human saliva, and their viscosity measured along different shear rates (0.001-100 s^{-1}) in a rheometer. The density was measured using an electronic densitometer. For the descriptive sensory analysis, a trained panel (n = 11) evaluated the body / unctuousness, astringency, alcohol, acidity and bitter taste. The results show a correlation between the density of the samples with saliva and body perception in the absence of ethanol ($R = 0.971$, $p = 0.029$). Wine formulations containing tannins were the most viscous, in correlation with the sensation of astringency ($R = 0.855$, $p = 0.030$). No correlation was found between viscosity and body perception, due to the sensory saturation given by the phenolic components. Therefore, these results sustain the understanding of the wine texture through instrumental measures, in which the presence of saliva has been revealed as a key factor to be considered. Studies concerning mechanistic modelling using commercial wines and experimental validation are in course.

An interesting approach to find grape quality markers

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It is well known that, wine sensory properties unquestionably depend on grape features. However, the relationship between grape and wine quality, is almost unknown. In this context, the aims of the present work were 1) produce wines with grapes harvested at different maturity points, 2) sensory characterize the wines, 3) study the effect of grape maturity on wine sensory properties and 4) find quality markers in winegrapes driving wine quality by correlating chemical variables (amino acids and phenolic compounds) of grapes and flavor attributes of final wines.

For it, Moristel grapes were collected during two consecutive harvests at different stages of ripening (one week between stages). Harvest took place at four different maturity points in 2016 and three points in 2017. Grapes were handpicked at commercial ripeness except for the first point of maturity. Wines were elaborated in 75-liter tanks, in triplicate, and in parallel, grape extracts were obtained. The wines were sensory described by the rate-all-that-apply method and the effects of grape maturity on the sensory features of wines were evaluated.

Results interestingly showed that, grapes prematurely (first point of maturity) harvested yielded high oxidation nuances (acetaldehyde-related), significantly correlated with total and free acetaldehyde concentrations quantified by HPLC-UV/VIS. Whereas, wines from grapes harvested at intermediate commercial points of ripeness were described with high levels of fruity aromas (red and black fruit). Further, correlations between oxidation features of wines and the composition of grape extracts were established.

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Influence of Mepanipyrim and Tetraconazole on the byosinthesis of volatile compounds through the phenylalanine Ehrlich pathway

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Fungicide residues and nitrogen sources may promote changes on the metabolic response of yeasts and consequently, alter the sensory quality of wines. For this reason, a better knowledge of amino acid x substrate x fungicide interactions during the alcoholic fermentation is mandatory.

Fermentations of synthetic musts inoculated with *Saccharomyces cerevisiae* var. *bayanus* (Lalvin T73™) were performed (n=6) at laboratory scale, under controlled conditions using phenylalanine as the unique source of nitrogen (160 mg/L). Previously, musts were spiked separately with Mepanipyrim and Tetraconazole commercial formulations at concentration levels of twice their respective maximum residual limits on grapes established by EU legislation. A control assay, without fungicides, was also carried out for comparative purposes.

Since the formation of higher alcohols is closely linked to the metabolism of amino acids by yeasts, through the Ehrlich pathway, this study was focused on the analysis of 2-phenylethanol production by direct injection on a GC-FID [1]. To better understand the variations and evolution of this alcohol, the concentration of its precursor, phenyl acetaldehyde, and its subsequent derivative, 2-phenylethyl acetate, were determined by liquid-liquid extraction [2] and their detection was carried out by GC-FID. Additionally, phenylalanine consumption was evaluated by HPLC-DAD [3].

Although, no statistical significant differences with respect to the volatile compounds were observed in the final wines, the presence of fungicides seems to accelerate this pathway. Phenylalanine consumption also supports this hypothesis.

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Effect of closure and storage temperature on the organoleptic characteristics of Torrontés Riojano (Mendoza–Argentina) wines conserved in bottle during 18 months

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During their storage, wines in the bottle, due to the type of closure and temperature, under go changes in their organoleptic characteristics. The aim of this study was to evaluate the effect of temperature and type of closure on the sensory characteristics of Torrontés Riojano wine (Mendoza, Argentina) during 18 months of conservation. Wine making was carried out on an industrial scale under standard protocol. Wine obtained was bottled (in-triplicate) considering the closure type [screw-cup (R), cork (C), synthetic (S)] and temperature (15ºC-25ºC). The sensory analysis was carried out with 10 judges trained at 6, 12 and 18 months of conservation. The attributes colour-intensity (IC), greenish-hue (MV), yellowish-hue (MA), brownish-hue (MP), aromatic-intensity (IA), fresh-fruit (FF), linalol (Li), floral (F), acidity (Ac), bitterness (A), herbaceous (He), azahar (Az), chemical (Q) and rusty (Ox) were evaluated. The sensorial results were analized by a mixed lineal model (MLM-software-R), principal component analysis (ACP-software-SensomineR) and confidence ellipses. At 6 months R, S and C (15ºC) showed the highest IA, FF, Li and MV; S and C (25ºC) showed the highest IC, MA and Ox; and R (25°C) showed an intermediate behaviour with a decrease in FF, Li and MV compared to R at 15°C. At 12 months, were observed differences between both temperatures, but not by closure types. At 15°C Az, Li and FF were higher and IC, MA, IA, Q and Ox were lower than at 25°C. At 18 months, the treatments at 25°C showed higher IC, but R and C showed higher MA and Ox and lower MP and He than S; C, R and S at 15°C, showed no difference. Conclusion: the storage temperature was the factor that allowed to separate the treatments. At 15°C the wines presented higher FF and F (typical descriptors of Torrontés Riojano); at 25°C, higher IC, MP, MA, Ox and He and loss of varietal character.

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Red wines quality judgements prediction by experts and wine consumers using a trained panel

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The goal of this study is to use the sensory opinions given by a trained panel for the prediction of the wine quality assessments done by experts and consumers. Some studies use the descriptors from a trained panel to relate them to the perceived quality of the wines. There is a need to adapt the obtained information depending on the type of judges and the aim of the research. Twenty-nine oak aged red wines of DOCa Rioja were described for their orthonasal and mouthfeel characteristics by a trained panel. The overall quality given by wine experts' panel and the consumers' punctuation provides the quantitative assessment of the wines. The lower, average and higher scores provides three groups of wines. Logistic regression was applied twice to detect the predicting terms, first with-in the group with average and lower quality scores, and secondly the higher versus the average wines. For the prediction of consumers' scores, the same aroma factor is significant to differentiate between lower and averages wines and from higher and average wines. In contrast, for the prediction of experts, is more complex using more terms and different in order to differentiate wines from average and low, and high from average group. Some aroma terms and persistence are the essential attributes to distinguish between lower and average wine but astringency and another aroma terms are significant for differentiate average and higher quality wines by experts. These models has showed a good capacity of adaptation in order to predict the quality valuations of experts and consumers: In all cases, the predictive models are around 80 % for the models that differentiate wines of relative low vs. medium and medium vs. high quality judgements.

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Sensory characterization of ultrapremium Spanish wines and its relation with the structure

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Ultra-premium wine are distinguish by their highest level of extrinsic and intrinsic quality. The complexity of in mouth sensations on red wines is a topic of great interest to understand and determine those sensory terms that contribute to the structure of the wine perceived in mouth. Little studies refer to Spanish ultra-premium sensory space and their wine structure. The aim of this research is to define the sensory space of the flavor of these wines and to explain the relation of the aromatic attributes and of tactile sensation in mouth to the structure of the wine. Hierarchical Cluster Analysis of the sensory data given by wine professionals provides seven representative wines out of twenty-one Spanish ultra-premium wines. The expert panel evaluates by quantitative descriptive analysis the seven Spanish red wines and PLS was executed. The bootstrap resampling procedure was applied to 5000 subsamples to obtain the statistical significance of the model, and a blindfolding process detect if the model specification was adequate (Stone-Geisser test: Q2 must be positive). The PLS results showed that the principal connected attributes with the structure are positively correlated with the in mouth attributes of persistence, mouth-coating fleshy, oily and sticky whereas the attributes of aroma contribute moderately being the red and black fruits, spicy, toasted those who have a major weight.

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Aroma variability induced by individual esters in simple solutions is not observed in complex mixtures representing red wines

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Ethyl esters are a group of volatile compounds that impart fruity notes to wine. Up to this date, around 14 ethyl esters bearing slightly different specific aroma descriptors have been identified. Some authors have suggested that individual components have crucial relevance in defining the fruity notes finally perceived in red wines. We, however, hypothesise that such sensory differences perceived in simple solutions cannot be perceived in a complex mixture such as red wine, so that the 14 compounds can be replaced by mixtures of just 3 or 2 components without losing sensory information.

In this work, a mixture of 14 esters (V1), formed by 5 lineal and 9 branched esters, was prepared in buffered solution (pH 6) at concentrations found in wines [1]. In parallel, other simpler and isointense vectors with 6(V2), 4(V3), 3(V4), 2(V5-V6) or 1(V7) individual esters were generated. The iso-intensity of the seven vectors was evaluated using a 1-butanol reference scale [2]. Then, ten different reconstituted wines were prepared mixing the volatile composition of a young red wine or an aged oaked red wine, the non volatile matrix corresponding to each one and the selected ester vector.

Triangular tests with 15 semi-trained judges demonstrated that all the aroma variability induced by individual esters (observed in simple solutions) can be in fact be well represented by three (in a young red wine) or even one (in an aged oaked red wine) compound, which strongly questions the alleged importance of some minor esters. This confirms the need to work with complex mixtures similar to the target product to evaluate the ability of certain compounds to modulate the aroma properties.

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Poster Session B

Light effect on the establishment of embryogenic cultures of *Vitis vinifera* L. Portuguese grapevine varieties

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Light is one of the most important environmental factors affecting plant growth/development. The biologically active radiation is included in the spectrum from approximately 300 to 800nm including UV-light (below 400 nm) and visible light spectrum (between 400 and 710nm). The importance of light in plant regeneration has been reported in several plant studies including *Vitis vinifera* L.[1]. The induction of somatic embryos in grapevine is dependent on many factors, from genotype to growing conditions. In this study, the effect of light intensity and duration of exposure on *calli* formation and embryo induction using anther culture was explored. Anthers of the grapevine varieties "Viosinho" and "Malvasia Fina" were collected at the phenological stage G (separate clusters) and plated in culture media, supplemented with different concentrations of growth regulators: 2,4-dichlorophenoxyacetic acid (2,4-D), 6-b*enzyl-aminopurine* (BAP) and beta-naphthoxyacetic *acid* (NOA). Through the first seven weeks of culture, explants were exposed during 1 h/week to various wavelengths radiations: UV-light (350 nm) and visible light (blue - 450 nm and red - 600 nm). Grapevine varieties behaved differently to light exposure. "Viosinho" presented 96% and 82.6% of *calli* induction in response to 450 and 600 nm radiation exposure, respectively, in culture medium containing NOA. On the contrary, "Malvasia Fina" didn't respond favorably to the light exposure presenting a very low *calli* induction rate for all wavelengths and culture media tested.

Up till now grapevine varieties were receptive to the light treatments, considering primary *calli* proliferation; however, no somatic embryos were produced. Nevertheless, new experiments are underway for further investigation.

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Phenolic characterization of *Vitis vinifera* intraspecific hybrids from Monastrell and their wines.

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Monastrell is a very late variety in terms of sprouting and ripening, but is well adapted to the agroecological conditions of southeastern Spain, however, despite its high phenolic composition, its thick skin makes it difficult to extract it during the winemaking process (Gómez-Plaza et al., 2008), it is therefore necessary to adopt some kind of measure that allows us to take advantage of its full potential. On the other hand, given the consequences of climate change, it is urgently necessary to obtain new varieties through intraspecific crossings using Monastrell as a parental, which must show a good adaptation to our climatic conditions and also obtain a high content of phenolic compounds.

A collection of ten hybrids from crosses from Monastrell x Cabernet Sauvignon (MC) and Monastrell x Syrah (MS) were selected since 2003 for its high phenolic content. Also, the harvest time was taken into account for the selection of the crosses in order to obtain hybrids adapted to different environments.

The results showed how the hybrids analysed and their corresponding wines could be classified in five different time harvest beginning in early August and ending in early October. The phenolic content was higher in the selected hybrids than their parental, being the hybrid MS10 the one that obtained a greater concentration of anthocyanins and flavonols, the hybrid MC98 the one that obtained a greater concentration of tannins and finally, the hybrid MC111 the one that obtained a greater concentration of stilbenes. Nowadays, MS10, MC98 and MC80 have been requested European registration and protection.

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Grape color variation involves genetic and micro-environmental changes that alter berry phenolic and aromatic composition

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Black- and white-berried grapevine cultivars are distinguished by their ability to accumulate anthocyanins in the berry skin. To assess possible side-effects of color variation in berry development and composition in a near-isogenic background we compared white-berried somatic variants (WV) to their black berry ancestors in Garnacha and Tempranillo cultivars. Absence of anthocyanins correlated with lower berry temperature at daytimes in WV. At transcriptome level, besides anthocyanin accumulation-related genes, transcripts encoding enzymes involved in flavonoid backbone biosynthesis and modification were down-regulated from veraison in WV skin. Genes mapping on hemizygous genome regions in WV were downregulated as well irrespective of berry tissue or developmental stage. Light-responsive genes including flavonol and monoterpenoid biosynthesis genes were upregulated in WV from veraison. In agreement, flavonol partitioning was altered and tri-hydroxylated forms were practically absent in WV, whereas higher levels of specific volatile monoterpenoids and their soluble precursors were detected in WV pericarp. Interestingly, levels of the stress-related GABA were lower in WV skin, whereas Phe and Tyr precursors of phenolic compounds tended to be higher in WV pericarp. Greater differences were observed in Tempranillo than in Garnacha evidencing genetic background-dependent effects. These results indicate that the grape color locus directly controls the metabolism of colorless flavonoids, whereas additional alterations in grape quality compounds are established in a cultivar-dependent manner in response to alteration of the berry microclimate caused by the absence of anthocyanins.

Agronomic and qualitative behavior of 9 preselected clones of Prieto Picudo Oval red variety in the A.O. Tierra de León

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The advance in the development of many wine-growing areas with prestige and recognition of their wines usually has its origin, among other factors, in the cultivation of autochthonous varieties well adapted to the area [1]. On the other hand, there are other varieties cultivated marginally but well adapted to their areas of origin, as is the case of the Prieto Picudo Oval red variety in the Appellation of Origin Tierra de León [2]. This variety is located mainly in the southeast of the province of León and in the north of the province of Valladolid.

The objective of this work is the productive, vegetative and qualitative characterization of 9 preselected clones of the Prieto Picudo Oval variety. The clones have been cultivated on a comparison plot located in Melgar de Abajo (Valladolid), within the A.O. Tierra de León. The study period covered 4 years (2014-2017). The different clones were grafted between 2007 and 2008 on the Richter 110 rootstock, which were planted the previous year. The planting frame is 2.6 x 1.2 m. The plants are pruned in bilateral Royat system with thumbs with 2 buds.

The results obtained show differences between the 9 preselected clones, showing some variability in the aspects studied. The choice of a certain clone will depend on the productive and qualitative objectives pursued. These results are a good basis for the selection of some of the clones included in the present work.

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A missense mutation in the MADS-box gene VviAGL11 is responsible for seedlessness in table grapes

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Seedlessness is greatly prized by consumers of fresh grapes. While stenospermocarpic seed abortion determined by the SEED DEVELOPMENT INHIBITOR (SDI) locus is the usual source of seedlessness in commercial grapevine (Vitis vinifera) cultivars, the underlying sdi mutation remains unknown. Here, we undertook an integrative approach to identify the causal mutation. Quantitative genetics and fine mapping in two 'Crimson Seedless' (CS)-derived F1 mapping populations confirmed the major effect of the SDI locus and delimited the sdi mutation to a 323-kb region on chromosome 18. RNA-seq comparing seed traces of seedless and seeds of seeded F1 individuals identified processes triggered during sdi-determined seed abortion, including activation of salicylic acid-dependent defenses. The RNA-seq dataset was investigated for candidate genes and, while no evidence for causal cis55 acting regulatory mutations was detected, deleterious nucleotide changes in coding sequences of the seedless haplotype were predicted in two genes within the sdi fine mapping interval. Targeted re-sequencing of the two genes in a collection of 124 grapevine cultivars showed that only the point variation causing the Arg197Leu substitution in the seed morphogenesis regulator gene AGAMOUS-LIKE 11 (VviAGL11) was fully linked with stenospermocarpy. The concurrent post-zygotic variation identified for this missense polymorphism and seedlessness phenotype in seeded somatic variants of the original stenospermocarpic cultivar supports a causal effect. We postulate that seed abortion caused by this amino acid substitution in VviAGL11 is the major cause of seedlessness in cultivated grapevine. This information can be exploited to boost seedless grape breeding.

Characterization of deletions causing berry color variation in Garnacha and Tempranillo

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Gray and white somatic variants that can be the base of new cultivars occasionally appear in some black-berried grapevine cultivars. Genetic and molecular studies have associated color loss to the emergence of deletions at the grape color locus located on chromosome 2 in heterozygous colored cultivars carrying a functional and a null allele. Depending on the size of the deletions, side-effects additional to the loss of pigmentation capability may appear in these variants. In this study, we developed a SNP-based chip to evaluate along chromosome 2 the extension of hemizygous deletions in grape color variants through loss of heterozygosity analysis. These markers were used to characterize white and gray isolates originated from Garnacha Tinta and Tempranillo Tinto and collected along the Ebro valley (NE Spain). Two main deletion classes were detected in Garnacha Blanca correlating with the geographical origin of the accessions, while Tempranillo gray berry variants showed higher variation. Comparative genomics of Garnacha variants after whole-genome re-sequencing was addressed to understand the mutational mechanisms generating color variation. The results show that these deletions are generally associated with more complex genome rearrangements. Additional structural variation between independent lines of Garnacha Blanca likely emerged in different ancestral clonal lines of Garnacha Tinta. These results can be exploited for the selection of color variants best suited for the development of new cultivars and can help to detect rearrangement hotspots in the grapevine genome.

PollenCounter, a new tool for grapevine pollen viability automatic phenotyping

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Pollen quality is an important component of plant reproductive behaviour. A limited pollen viability may compromise grapevine pollination and fecundation, lowering the number of seeds per berry, the fruitset rate and the number of seeded berries in the cluster, which ultimately might reduce vineyard yield. Nevertheless, and in spite its relevance, the information available is limited to a low number of grapevine cultivars. In this regard, both basic and applied research will benefit from the development of an objective, accurate and rapid cost-effective method for the high-throughput evaluation of pollen viability. Here, we introduce *PollenCounter*, a new image-based tool for the high-throughput phenotyping of pollen viability in grapevine. This tool splits the RGB images of stained pollen grains into its primary channels to perform an independent counting of viable and total (viable + non viable) pollen grains to calculate pollen viability. *PollenCounter* has been successfully validated in pollen samples from diverse grapevine cultivars, providing accurate, precise and fast information. The in-depth analysis of a collection of table- and wine-grape varieties revealed for the first time a wide range of diversity in pollen viability variability in the cultivated grapevine, from cultivars having very low pollen viability to others with a very low presence of non-viable pollen grains. In addition, a large variation was observed among a set of Tempranillo Tinto clones. Due to its high precision and accurateness, *PollenCounter* is expected to substitute time-demanding manual processes for pollen viability evaluation, speeding-up studies aimed to unravel the physiological and genetic mechanisms affecting this trait in grapevine.

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On the causes of bunch compactness: Characterization of the reproductive performance of grapevine cultivars

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Bunch architecture and number of berries are the two the main causes of bunch compactness in a multi-cultivar frame. In order to deepen berry number establishing and bunch compactness origin, the reproductive performance of a set of 120 wine grape and table grape cultivars was studied in two consecutive growing seasons.

Genetic, physiological and environmental factors interact in grapevine to determine the flower number in the inflorescence and the fruitset rate. Abnormal behavior in the reproductive performance may cause coulure (excessive fall of flowers) or millerandage (excessive presence of seedless berries and/or live green ovaries (LGOs)), which may affect yield. Measures of reproductive performance were taken in ten inflorescences/bunches per cultivar and included flower number per inflorescence, berry number per bunch, fruitset (%), coulure and millerandage indices. Different methods were used to estimate fruit set, millerandage and coulure indices. Fruitset average values among all the cultivars was 46-48% in 2016 and 41-42% in 2017. The whole range of variation for fruitset among cultivars was very high, more than 90% every year. Within cultivars, differences in fruitset between years ranged from 0 to 46%, with an average difference around 9-10%. Coulure and millerandage indices also showed great variability among cultivars, reaching the whole range of variation, while were stable between seasons (difference average: 1 unit).

The results allow establishing a preliminary classification of a large number of cultivars according to their reproductive performance by using clustering analysis. Besides, the huge variability found constitutes a very suitable base for the study of the genetic processes involved in the grapevine reproductive performance and its possible relationship with bunch compactness.

Bioactive compounds content in the grape skin and relationships with skin colour

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Grapes are one of the most consumed fruits in the world and they also stand out as a source of phenolic compounds which are important to human health. Results in the literature suggest that these and other bioactive components as melatonin present in grapes, can act as powerful antioxidants that are capable of scavenging free radicals in cells [1,2]. Previous studies showed that melatonin is associated to the anthocyanins production (present in grape skin and responsible for the red color). The geographical and climatic factors as well as cultural practices are related with the variations in the content of these compounds in the grape[3].

The aim of this work is to study the content of melatonin and phenolic compounds in the grape skin and their distribution in diferent geographical regions.

In the present study, melatonin and several phenolic compounds such as resveratrol and catechin were determined in 515 varieties from different geographical origins, with different colours and other characteristics. Extraction was performed from grape skins and the extract was analyzed by HPLC.

The results have shown high concentrations of melatonin in red grapes (dark red violet and blue black skin colour), whereas it was not detected in 33% of total grapes analyzed, being most of them green yellow grapes. These data will be used in further studies of genetic basis of these traits in a genome wide analysis.

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Key words: melatonin, phenolics compounds, colour, grape skin.

Flavan-3-ols in seeds of a Grenache x Tempranillo progeny: influence of polymerization on antioxidant activity

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Flavan-3-ol compounds are important secondary metabolites with high antioxidant activity and responsible for bitterness and astringency of food products. This compounds are mainly in form of polymers, and the degree of polimerization is an important factor that affect grape and wine properties. The aim of this study was to analyze the seed flavanol content from a breeding population obtained by crossing two major Spanish wine varieties, Grenache and Tempranillo, and the influence of polymerization on antioxidant activity. Polymerization was determined by phloroglucinol reaction followed by HPLC-DAD-MS analysis, and antioxidant activity were determined using DPPH reagent followed by spectroscopy.

Chromatograms showed a similar profile for all samples and the same three flavan-3-ol monomers and its adducts were detected, (+)-catechin (CA), (-)-epicatechin (EC) and (-)-epicatechin-3-O-gallate (ECG). The most abundant compoud was EC adduct while the least was CA adduct. Significant differences were found between parental varieties, CA and EC were higher for Grenache, while medium degree of polimerization and percentage of galloylation were higher in Tempranillo. Hybrid population presented continuous variation for all compunds and parameters derived. Comparing red and white hybrids, ECG adduct was higher in red hybrids. Antioxidant activity showed a strong positive correlation with each compound, and negative correlation with medium degree of polimerization.

Grenache and Tempranillo differ in flavanols content and polimerization. The study of progeny revealed that flavan-3-ol compounds are quantitative traits with a transgressive inheritance and a determining factor in antioxidant activity. Finaly, antioxidant activity seems to be mainly afected by monomers content, specially CA and EC.

Keywords: Vitis vinifera, grapevine, mDP, phenolic compounds

An integrated approach for wine authenticity: the case of Alvarinho

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Wine authenticity, certification and origin has become a growing priority amongst consumers and producers, especially for wines whose commercial value is associated to a denomination of origin. Single Nucleotide Polymorphisms (SNPs) represent the most basic and abundant form of genetic sequence variation being one of the most reliable forms of varietal identification. Because, DNA-based methods are not suitable for geographical determination, wine/must origin has been assessed by the strontium $^{87}Sr/^{86}Sr$ isotope ratios determination, imposing their determination in the soil fraction in order to extrapolate to the wine samples. This work aimed to test a wine integrated authenticity system, guarantying: 1) grapevine varietal identification in wine, based on varietal SNP information, by High Resolution Melting (HRM) analysis and; 2) geographical provenience based on $^{87}Sr/^{86}Sr$ isotope ratio in soil and wine samples. This study was conducted using the grapevine variety Alvarinho. Three different locations were studied, belonging to two different Appellations'. Rock, soil, and wine samples were collected. Three genes of the anthocyanin pathway, the flavanone 3-hydroxylase (*F3H*), *U*DP glucose–flavonoid 3-O-glucosyl-transferase (*UFGT*), and the leucoanthocyanidin dioxygenase (*LDOX*) were used for SNP identification. HRM testing was based on previous designed assays [1,2]. All HRM were validated through Sanger sequencing and confirmed the variety identification capacity of the assays. The $^{87}Sr/^{86}Sr$ isotope ratio revealed to be consistent in rock and soils from the same origin, however when the wine was tested it was clear that the values decrease substantially and no clear relation with the soil origin was found.

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On the causes of bunch compactness: Phenotypic, hormonal and genomic variation among Tempranillo clones

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Previous studies showed that the number of berries is a major component of the compactness level of the grapevine clusters. Variation in number of fruits is regulated by events occurring in the fruitset, but also before, during the flower formation and pollination, through factors like the initial number of flowers or the gametic viability. Therefore, the identification of the genetic bases of this variation would provide an invaluable knowledge of the grapevine reproductive development and useful tools for managing yield and cluster compactness. We performed the phenotyping of four clones (two compact and two loose clones) of the Tempranillo cultivar with reproducible different levels of cluster compactness over seasons. Measures of reproductive performance included flower number per inflorescence, berry number per cluster, fruitset, coulure and millerandage indices. Besides, their levels of several hormones during the inflorescence and flower development were determined, and their transcriptomes were evaluated at critical time points (just before the start and at the end of flowering). For some key reproductive traits, like number of berries per cluster and number of seeds per berry, clones bearing loose clusters showed differences with the compact clones and differed from each other, indicating that each one follows different paths to produce loose clusters. Variation between clones was observed for ABA and Gibberellins levels at particular development stages, which could be related to phenotypic differences. Likewise, various changes between clones were found at the transcriptomic level, mostly just before the start of flowering. Several differentially expressed genes between one of the loose clones and the compact clones are known to be over-expressed in pollen, and many of them were related to cell wall modification processes or to the phenylpropanoids metabolism. We also found polymorphisms between clones in candidate genes that could be directly involved in the variation of the compactness level.

Uncovering Northeast Portugal grapevine's varietal legacy

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The northeast Portugal comprises the wine denominations 'Douro', the oldest demarcated and regulated wine region in the world, and 'Trás-os-Montes'. There are more than one hundred grapevine cultivars registered as suitable for wine production in these regions (ministerial-orders number 1204/2006 and 383/2017), but only a few are actually used for winemaking. In this sense, resorting to a much higher number of varieties could be an important step to take advantage of all the potential of these regions grapevines' biodiversity. The conservation of these grapevine genetic resources promotes a greater products' diversification and it can be considered a strategic support in the improvement and valorization of 'Douro' and 'Trás-os-Montes' DOC wines.

Hence, the main goals of this study were to prospect and characterise, through molecular analysis, a broad representation of grapevine cultivars patrimony of 'Douro' and Trás-os-Montes' regions.

Leaves of about 300 plants were sampled in seven different old vineyards to proceed to microsatellite genotyping with the SSR markers recommended by the *Organisation Internationale de la Vigne et du Vin*. The SSR profiles were compared to those of SSR databases. A high percentage of the genotypes detected correspond to minor and neglected varieties and a considerable number to unknown genotypes.

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Water Use Efficiency Ranking as selection criteria of Tempranillo in a long–term trial.

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Grapevine has a wide distribution in arid and semi-arid regions. The IPCC concludes that in the future these areas could be severely affected by extreme drought episodes. Then, its expected a growing competition for the use of available freshwater. To address these challenges, it will be necessary to use new genotypes with high efficient in the use of water. At leaf level, intrinsic water use efficiency (WUE_i) has been described as the ratio between net photosynthesis and stomatal conductance (A_N/g_s). In this work, the WUE_i as selection criteria in two collection of Tempranillo genotypes was tested. We carry out the experiment under fields conditions in two different locations: Rioja (Instituto de las Ciencias del Vino y la Viticultura Logroño, Spain) and Haro (Roda Wine Cellar, Spain). Gas exchange had been measured in two consecutive years during veraison phenological stage. To compare the WUE_i under different soil water availability conditions, WUE_i was plotted against g_s obtaining a regression line of very high r^2 coefficient which express the known dependency of WUE_i from g_s. To evaluate the performance in WUE_i between genotypes at a given g_s, the residuals of this relationships associated to each genotype were calculated. Our results showed a significative correlation (r^2=0.45, p-value<0.05) between years at similar hydric stress level. Its mean that genotypes could be ranking by their residual value. However, this relative position could be affected by the water status of the plant. To the stability of the ranking method, its necessary that each genotypes evaluated has similar water regime, but if not reached, as in the ICVV experimental location, the ranking was not conserved. In conclusion, the present work proposed a new screening method to make a selection based on the WUE_i.

Influence of berry size on the enological composition of Pinot Noir New Zealand wines

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Berry size is a relevant trait for enological quality, and it is usually accepted that smaller size results in higher skin to pulp ratio and higher polyphenolic content, rendering higher quality wines. However, there are contradictory reports regarding this issue. In this work were collected in the same plot bunches of different sizes of Abel Pinot Noir Clone in Marlborough Region, New Zealand. Four different bunch ranks were created attending to their weight: less than 100 g. per bunch, between 120 and 170 g, between 170 and 240 g and more than 240 g per bunch. Harvest was conducted when all bunches reached phenolic ripening (at around 21 Brix degree). Microvinifications were elaborated with 4 replications for all sets, except to for the greater size group in which 3 replications were done. Wines were analyzed by OIV official methods, and the parameters studied were pH,º brix, total acidity, NH4+, Yan, Amino acid profile content, Color Intensity, Tonality, Total Phenolic content, Monomeric and Total Anthocyanins. Regarding berry characterization a subsample of 200g berries was taken of each category and mean berry weight, number and mean seed weight per berry data was collected. Besides berry length, diameter, mass and volume and seed mass were measured with Image analysis tools. Results showed that mean berry weight, number seeds per berry, berry mass, berry length, NH4+, amino acid profile and Yan presented greater statistical values as long as bunch weight category was higher, whereas total phenolic content, monomeric and total anthocyanins showed higher statistical values as long as bunch weight rank was lower. This result suggests that small berry size conduct to better quality wines, but the evaluation of wine chemical content needs to be completed with sensorial analysis.

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Characterization of Graciano x Tempranillo genotypes and associations with flower and berry parameters

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Consumers demand for different and high quality wines has been answered by market introducing foreign varieties. An alternative strategy could be to obtain cultivars by the genetic improvement of traditional varieties selected for better adaptation to climate change.

In this context, the aim of this study was to evaluate 12 genotypes selected from a progeny of Graciano x Tempranillo, and to assess the relation between flower and berry parameters and wine composition. Selection of genotypes was based on data from 3 different vintages including agronomic traits as well as quality traits. Out of the 12 genotypes studied, 7 were considered early-ripening, compared to Tempranillo, and 5 late-ripening. Microvinifications were conducted in 2017 with 3 replications for both set of selections and the parental genotypes. Wines were analyzed by OIV official methods, for the standard parameters. For the analyses of berry parameters data were taken of two different harvests by measuring berry length, diameter and shape. Flower parameters; length, diameter and shape of pistil and ovary, were measured by digital tools. Results suggest that late-ripening genotypes differed significantly from Tempranillo, having lower values of malic acid, pH, Cielab index, berry length, diameter and size. Based on those results, they could be and interesting alternative in the context of climate change, depending on sensorial analysis. Besides, late-ripening genotypes had significantly lower values of IPT and IC in comparison with early-ripening ones, and higher Cielab parameter index. A correlation matrix showed that berry diameter was significantly negative correlated with anthocyanin content, IPT and IC, suggesting that small berries lead to higher quality. Also, a significant positive correlation between ovary and berry shape, and IPT was found, showing associations that would need to be corroborated by genetic analyses.

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Study of candidate gene variants pleiotropically controlling berry shape and other reproductive traits in grapevine

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Diversity in berry size and shape has been selected during grapevine domestication. While spherical shapes are more frequent in wine grapes to increase the proportion of secondary metabolites accumulated in the berry skin, larger berries with striking forms are attractive for table grape consumers. To gain insight on the molecular mechanisms generating such diversity, here we analyzed the putative involvement of candidate gene variants identified in contrasting materials of different origin. On one hand, we studied a somatic variant with more elliptic berries and enlarged seeds emerged in the wine cultivar 'Prieto Picudo'. An RNA-seq transcriptomic comparison identified a candidate *GLUTAMATE RECEPTOR-LIKE* (*GLR*) gene that is over-expressed in the berry shape variant compared to the ancestral 'Prieto Picudo'. Sequencing and RT-qPCR confirmed that 'Prieto Picudo' is heterozygous for this gene and only one allele is over-expressed in the somatic variant, which suggest that a heterozygous *cis*-acting misexpression mutation in the *GLR* could cause the variant phenotype. On the other hand, a region in chromosome 5 controlling berry shape and size variation in table grapes was identified after a QTL analysis in a 'Red Globe'✕'Crimson Seedless' F_1 population. One allele from each progenitor associated with the development of enlarged berries and with decreased plant fertility. For one gene co-localizing with the QTL, transcriptomic studies identified prominent over-expression in several reproductive tissues of F_1 individuals harbouring the associated alleles. Supporting its involvement, this gene encodes a putative member of the SWR1 chromatin remodelling complex that controls reproductive development in plants. PacBio sequencing of BAC clones for the two alleles in 'Crimson Seedless' identified polymorphisms that could be related with the over-expression. These studies identified novel putative gene functions in plants and show how independent dominant misexpression mutations could have been selected in grapevine associated to the emergence of new interesting reproductive traits.

Recovery and valorisation of ancient varieties grown in high-altitude vineyards of the Spanish Pyrenees

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This project aims to recover ancient grapevine varieties in the Biescas area (Huesca, Spanish Pyrenees), after studying its molecular profile and agricultural and oenological potentials. The molecular analysis will be performed with eight microsatellites markers with high discriminant power and the profiles will be checked in national and international databases to determine the varietal identity. The oenological aptitude of these varieties will be evaluated with micro and standard vinifications carried out in Bal Minuta Cellar. To expand the commercial vineyard, a new plantation is planned in a currently abandoned field located near the recovered village of Barbenuta. Due to the mountainous climatic conditions, the high slope gradient of the field and the characteristics of the drainage area, the processes of water soil erosion and hydrological connectivity (HC) will be studied by means of field assessments and numerical modelling. The aggregated index of HC (AIC) will be run before and after plantation establishment. In this landscape, the abandoned and recovered fields are intermingled with patches of forestry areas where high walls and stone walls are frequent and influence on the runoff pathways.

This initiative aims to recover abandoned fields, to identify ancient varieties and to valorise the potential of these varieties cultivated in vineyards located around 1200 m a.s.l. The achievement of these goals will allow a better transfer to the sector of the wines coming out of high altitude and will be of interest for the sustainability of the local economy.

The project “Recovery and valorisation of old mountain vine varieties (Tierra de Biescas - Huesca)” has the support of (i) the Government of Aragon (Spain) through aid for cooperation actions of agents of the agricultural sector (Rural Development Program 2014-2020), and (ii) the European Agricultural Fund for Rural Development (EAFRD - FEADER). Preliminary results will be presented during the Congress.

Selection and molecular characterization of 'Tempranillo' somatic variants with ripening features adaptable to climate change

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Global warming is threatening the sustainability of vitiviniculture in classical winegrowing areas. Increasing temperature and drought episodes in these regions hasten grapevine phenology and alter berry ripening, leading to excessively alcoholic wines that are often unbalanced in phenolic composition. Somatic variation that naturally emerges during the multiplication of elite wine cultivars can be exploited to face these detrimental consequences. In this manner, somatic variants completing ripening late in the season, when temperatures start to cool, would be suitable for quality wine production under warmer climate. Here, 494 accessions of the 'Tempranillo' cultivar held at the ICVV clone bank were screened under field conditions for variation in ripening period length trait. A pre-selection of 10 long (LRP) and 9 short ripening period accessions (SRP) showed similar flowering and onset of ripening (veraison) times, but differed in their sugar accumulation rates. The consistency of the ripening phenotype was confirmed using fruiting cuttings under greenhouse controlled conditions for two LRP and one SRP that in addition were balanced in yield and anthocyanin berry content. Considering that sugar content differences were established right after ripening onset, the transcriptome of these three accessions was compared in veraison berries (90% coloured skin surface) by RNA-seq analysis of pericarp. Clone-specific differential expression profiles were identified, suggesting that independent somatic mutations are responsible for the variant phenotype on each LRP. Variant calling from the RNA-seq data predicted one candidate dominant missense SNV specific of each LRP in genes coding for an ABC transporter and an Ubiquitin activating enzyme E1, respectively. Further genetic and functional work is required to validate these candidate mutations that could be useful in grapevine breeding programs dealing with adaptation of vitiviniculture to global warming.

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Poster Session B

Somatic embryogenesis for regeneration of improved Tempranillo Blanco lines from Tempranillo Gris variants

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Tempranillo Blanco (TB) is a white wine cultivar with high oenological performance that was developed from a bud sport of Tempranillo Tinto. Colour absence in TB berries is due to a catastrophic genome rearrangement, which affects gamete viability, compromises fruit set and reduces fruit production. We are using direct somatic embryogenesis methods that allow obtaining embryos of unicellular origin to regenerate new lines of TB with adequate productive features from Tempranillo Gris (TG) accessions. TG displays berries with grey colour due to the hemizygous deletion of *MYBA* genes only in the L2 meristem cell layer. In our case, direct somatic embryogenesis is carried out in selected TG lines with minimal deletions that do not compromise gamete viability to obtain unicellular embryos from the mutant L2 meristem cell layer. We used three independent accessions of TG (TRT, TRB and TRM) with different genome rearrangement levels. Somatic embryogenesis was induced from stamen filaments using thidiazuron (TDZ) and 2,4-dichlorophenoxyacetic acid (2,4-D). Four combinations of these growth regulators were assessed for somatic embryogenesis induction in a basal medium containing Nitsch and Nitsch salts and Murashige and Skoog vitamins. In addition, one explant group was dedicated to direct culture, whereas the others were maintained at different cold treatments before stamen excision and culture. We expect to obtain grapevine seedlings of different origins (both from L1 and L2 meristem cell layers), which will be characterized according to deletion of the functional allele of the colour locus. Further, since in vitro culture techniques may lead to genetic variations in plants through somaclonal variation, we will use flow cytometry to determine ploidy level of the regenerated plants. With this study we expect to obtain new TB lines, increasing the genotypic diversity and improving phenotypic traits for this relevant white cultivar.

Association analysis of grapevine traits related to reproductive performance

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Grapevine (*Vitis vinifera* L.) bunch compactness is becoming a key target for grapevine cultural management and for genetic improvement of table and wine grapes. Different environmental conditions, viticulture practices and genetic factors influence bunch compactness and its main components. In a multi-cultivar frame, the number of berries per bunch is one of the two main causes of bunch compactness (Tello et al. 2015), and that feature directly relates to the reproductive performance of a cultivar. A set of wine grape and table grape cultivars was studied in three consecutive growing seasons for traits related to the reproductive performance including flower number per inflorescence, berry number per bunch, fruitset, coulure and millerandage indices. In addition, a comparative transcriptome analysis of loose and compact clones of Garnacha Tinta and Tempranillo Tinto had generated a series of candidate genes for bunch compactness-related traits (Grimplet et al. 2017) which were sequenced in the same set of cultivars. Finally, a large association study was done with 114 cultivars, using phenotypic data obtained in three consecutive seasons and a set of 7032 single nucleotide polymorphisms (SNPs) identified by NGS in 183 selected genes (Tello et al. 2016). The study allowed detecting polymorphisms significantly associated with reproductive performance traits during the three studied seasons, in a few genes like MYB transcription factor 68 (VviMyb68). These genes and polymorphisms are interesting candidates for deeper analyses to validate the association results obtained in this work.

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Impact of vegetative balance on wine composition within Tempranillo vineyards in Ribera de Duero (Spain)

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The aim of this work was to study the effect of spatial variation of vigour and yield within the vineyard on Tempranillo wine composition. For this, 20 non-irrigated vineyard subzones (10 m x 10 m each) were selected in Ribera del Duero (North-Central Spain) considering different topographic and soil characteristics, to assure maximum variability in water and nutrient status across the sites. The vegetative expressions were monitored in 2016, and microvinifications were performed by duplicate in all subzones. Linear regression analysis showed no significant relations between vegetative balance and alcoholic degree, and total acidity of the wines. Nevertheless, vine vigour, estimated both as pruning weight or leaf area index (LAI), was positively correlated with wine pH. Increasing vine yield was associated with lower anthocyanin and tannin concentrations, and absorbance at 620 nm. Ravaz Index and balance of LAI to yield could estimate simultaneously pH and anthocyanin concentration of the wines. These preliminary results demonstrate the interest of vegetative balance indexes as indicators of potential quality of vineyards in Ribera del Duero.

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A tool to differentiate vineyard soils: Grape nitrogen composition

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In a small viticultural area, with similar climatic conditions modeled by the confluence of two rivers, the soil factor acquires special relevance in the typicity and characteristics of grape for winemaking. Nitrogen is the most important macronutrient, both for the grapevine optimum development and for the grape correct composition, which allows to carry out the alcoholic fermentation properly, since amino acids are the main source of nitrogen for yeasts, being also precursors of several volatile compounds, therefore nitrogen affects the wine aromatic quality.

The aim of this work was to study the influence of different soils on grape nitrogen content over four seasons. In order to determine this soil influence on must composition, Tempranillo (*Vitis vinifera* L. grafted on R-110) plots classified as *Fluventic Haploxerepts* (FH), *Typic Calcixerepts* (TC), and *Petrocalcic Palexerolls* (PP), situated in Uruñuela (La Rioja, Spain), were selected.

Grape amino acids and yeast assimilable nitrogen (YAN) allowed us to differentiate must samples from the three different types of soil, one of them, FH, more fertile and with more water reserve available and inorganic nitrogen, compared to PP, which had lower potential yield and TC, that had intermediate values. Moreover, grape amino acids and YAN concentration in must allowed us to differentiate, in each type of soil, samples of each season. In general, total amino acids content depended on the type of soil, season and their interaction. YAN concentration was more influenced by the soil-climate interaction and, to a lesser extent, by the soil. Ala, Thr and Tyr were the amino acids most affected by the soil type; Glu, Asp and Met were the most influenced by season; and Pro, His, Ser and Gly by soil-climate interaction. In conclusion, vineyards with lower amount of nitrogen available in soil produced musts with less nitrogen compounds content.

Effect of vineyard management and climatic conditions on productive, oenological and soil characteristics of a *Vitis vinifera* L. cv. Cabernet Sauvignon vineyard growing in semi–arid conditions during 5 consecutive seasons

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An experiment was carried out with the aim to evaluate the effect of vineyard management and climatic conditions on the productive and oenological behaviour of a Cabernet Sauvignon (*Vitis vinifera* L.) vineyard, together with the physicochemical characteristics of soil over 5 consecutive seasons. It has been reported that yield in grapevines growing in organic vineyard is lower than the conventional management, [1] while organic management results in important increases in soil organic carbon, which are critical for long-term fertility maintenance. [2] Based on this, the aforementioned parameters were evaluated in a Cabernet Sauvignon vineyard growing under organic, integrated and conventional management, located in Cauquenes, Región del Maule (Chile) during five consecutive seasons. Data about climatic variables was recording from an automatically weather station (AWS) located within the field trial.

The results indicated that bunch and berry weight, and number of berries per bunch were not affected by the vineyard management. Yield was higher in grapevines from conventional management than the organic one (5.95 to 4.83 t/ha), which was conditioned by the number of bunches per plant (44.5 to 33.7). Ravaz index was higher in grapevines under conventional management than the organic one (2.96 to 2.05). Any of the must oenological parameters were affected by the management, while wines from grapevines under integrated management presented higher total polyphenol index than organic samples (80.94 to 73.68). N and K soil content was the highest in the vineyard under organic management. Most of the productive parameters, must and wine oenological parameters and physicochemical characteristics of soils were more influenced by the season than the management. Therefore, season effect influenced productive, oenological and soil parameters more than the vineyard management in a Cabernet Sauvignon vineyard growing in semi-arid conditions.

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Agronomic classification between vineyards using NDVI and evaluation of the quality of their wines in A.O. Rueda.

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At present, there is a large group of consumers looking for differentiated products, so the food industry must offer several references to cover this demand. In wine sector, this scenario has favoured a very competitive market in which wineries seek to provide a wide range of products, with different characteristics among themselves. In this context, tools should be developed to obtain grapes with different qualities. In this way, remote sensing assisted by multiespectral imagery, is a good tool for monitoring vineyard [1] and making decisions to obtain different wines [2].

In this paper, a classification for vineyards belonging to Apellation of Origin Rueda has been established to determine the productive capacities of each one and to study their impact on grape quality. For the classification of these vineyards, open access multispectral images obtained from the SENTINEL-2A satellite were used to calculate the NDVI (Normalized Difference Vegetation Index), which provides information about the state of vegetative development and vigour of the vineyard. Based on NDVI, three vigour levels were stablished for vineyards classification (high vigour, medium vigour and low vigour), corresponding to three vineyards of Verdejo variety. Wine was produced following a common protocol, and finally a tasting was carried out to check if the differences between the vineyards with different vigour were perceptible.

According to the results obtained, the NDVI is a good indicator to classify vineyards, finding notable differences between the experimental treatments studied.

Acknowledgments

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Potential of the use of minority white grape varieties for the "Palo Cortado" sherry wine

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"Palo Cortado" is a complex sherry wines type as it combines the delicate bouquet of a biological aged wine (Amontillado) with the body and palate of an oxidative aged wine (Oloroso). The industrial elaboration of "Palo Cortado" wines, present some difficulties for the winemaker in the Jerez region (Spain), since this wine does not have an industrial proceeding for its elaboration. "Palo Cortado" wines appear spontaneously during the biological aging process in the "sobretablas" before the second classification or register in the winery. One of the most important factors in the formation of these wines could be the variety of grape and his grade of maturation. Currently, these sherry wines type are made with the Palomino Fino cultivar. However, several authors [1-3] indicated that in Marco de Jerez region was a great diversity of varieties, which allowed the emergence almost by chance of many types of wines with sensorial characteristics very typified such as "Palo Cortado" sherry wine type.

The aim of this research was to study the potential of Cañocazo, Castellano and Uva Rey white grape cultivars for the elaboration of "Palo Cortado" sherry wines. To carry out this study a physicochemical characterization of grape must from these cultivars was performed. In addition, the Palomino Fino cultivar (majority variety of the region) was also characterized as a control.

The three analyzed cultivars showed higher content in malic acid and glycerol than the Palomino Fino, as a consequence, wines made with these varieties would susceptible to develop malolactic fermentation process (lactic acid bacteria) that seems to be involucrated in the typical sensorial deviation of "Palo Cortado" wines.

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Microbiological and agronomic study of a restored soil vineyard with organic management

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Currently the vineyard soil microbiota is regarded as a key factor for wine quality and "terroir" character. The objective of this study was to analyse the culture-dependent microbiota of a restored soil of a vineyard under organic management, as well as its grape yield and quality. The sampling site was a vineyard located in Northern Spain (La Rioja), where some areas were assessed as degraded in 2015 due to their reduced vine growth and grape yield. In 2016 the following treatments were implemented: a) manure application, b) grass cover of oat and lucern, and c) grass cover of barley and faba bean. Both grass covers were managed as dry mulch. Samplings were performed during 2017 vintage. Five soil samples randomly distributed were taken in the areas of the soil treatments, in a non-degraded area and in an untreated degraded area with the same geographic location and organic management. Each sample consisted of four soil top-layer cores scattered in a range of 12 m and pooled together. On the whole, a total of 25 soil samples were analysed. Samples were stored at -80ºC until microbiological analysis. Soil microbial communities of yeast, nitrogen fixing bacteria, actinomyces and total aerobes were analysed and quantified. Grape yield and quality were assessed as number of bunches per vine, weight of produced grapes, weight of 100 berries and berry polyphenol content. Results showed that in all cases the vineyard soil was a large reservoir of yeast, and the main differences were obtained between the non-degraded area and the manure restored soil, which showed the highest grape production and the lowest polyphenol content per berry. Our results demonstrate the relevance of the vineyard soil as a yeast reservoir, and that given a geographic location, the vineyard management strategy plays a pivotal role in grape production and quality.

Tannin content and antioxidant capacity of five Greek red grapes varieties

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Tannins are responsible for important sensory and quality attributes of red grapes and wines, such as astringency, bitterness and colour stability. In this study berry attributes, skin and seed content of tannins and antioxidant capacity of 45 grape samples from five Greek Vitis Vinifera varieties, namely Mavrotragano, Mandilaria, Kotsifali, Agiorgitiko and Xinomavro were analyzed. For the total tannin estimation, the protein precipitation assay using bovine serum albumin was employed. Antioxidant capacity of the grape tannins was also determined, by the 2,2'-diphenyl-1-picrylhydrazyl radical scavenging method, in skin and seed extracts. Significant differences were observed in berry weight and the distribution of berry component mass in mature berries, % skin per berry and % seed per berry weight ratio, among the different varieties. Mandilaria and Kotsifali had the heavier berries while the higher contribution of skins and seeds in berry was observed in Agiorgitiko and Mavrotragano grapes respectively. Although, skins are in higher ratio from seeds per berry, in four out of five varieties, seed tannins have the higher contribution on total tannins per berry. According, to the results the higher seed and skin tannins concentrations were determined in Mandilaria and the lower in Xinomavro. Finally, varieties with high concentrations of tannins, Mandilaria and Mavrotragano, also demonstrated significant high values of antioxidant capacity.

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Multifactorial analysis of disease pressure and pesticide use in viticulture: reduction possibilities in Southwestern Europe

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Wine consumer preferences, along with the increasingly more restrictive regulations in pesticide use, represent a challenge for the European winegrowers and their viticultural practices, as well as for the winemaking sector competitiveness. The VINOVERT project integrates phytopathology, agronomy, economy and enology in order to develop feasible solutions for viticulturists in Spain, France and Portugal.

This study evaluates the possibilities in the pesticide reduction at vineyard scale and the effect of such reductions in the quality of the resulting wines. Thirty-two vineyards have been studied during 2017 and 2018, distributed in DO Rias Baixas, Penedès, and Bordeaux. The plots are commercially exploited by the growers without any previous recommendation. The measurements include: 1) Epidemiologic variables, such as the losses due to pests and diseases, integrated in the AIDB index; 2) Agronomical variables, regarding to yield and plot characterization, 3) Winegrower variables, summarized in the Treatment Frequency Index (IFT) and the Crop Protection Practices Index (IPP); and 4) Enological variables, such as pesticide residues in wine and organoleptic quality, by means of analyses and tastes.

The data obtained during 2017 in the three studied regions allow us to reveal some determining factors for the reduction in the pesticide use without a significant reduction in yield and wine quality. Multivariate statistical analysis are complemented with deeper analysis of some case studies for the comprehension of the observed variables. The contrasting meteorology in 2018 regarding 2017 will provide further deep insights.

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One year of combined effects of irrigation and early defoliation in five Spanish white grape cultivars in a particularly dry season.

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The early defoliation technique has been used successfully to improve colour in red grape cultivars, however, few information exists about their suitability on white grape combined with different vine water regimes. This study evaluates the effects of early defoliation (full bloom) on five white grape cultivars combined with two different water treatments (rainfed-R0 and R2, replacing 30% of ET0). The experiments were located in different production regions of Spain, in 2017: Viura (Extremadura), Verdejo (Castilla y León), Albariño (Galicia), Airén (Castilla La Mancha) and Tempranillo blanco (La Rioja). Preliminary results showed that, regardless of cultivar, yield was increased in R2 via highest cluster weight. Defoliation decreased cluster weight and yield in both R0 and R2 and the amount of yield lost depended on the cultivar. No clear irrigation and defoliation effects were observed on grape composition: sugars, titratable acidity, and malic acid, but tartaric acid was increased in R0 in several cultivars. The amino acids concentration of grapes, FAN, YAN and Ammonium in R2, were higher in Airen and Albariño and lower in Verdejo and Tempranillo blanco respert to R0. On the other hand, early defoliation decreased amino acids concentration on grapes regardless of irrigation. Total volatile composition of grapes was not influenced by defoliation, however, a trend to increase in R2 when defoliation was applied was observed in Albariño, Verdejo, Tempranillo blanco and Viura cultivars. Regarding volatile composition by families, a significant increase was observed in several cultivars. However, defoliation increased the ethyl esters concentration in Airen-R2. Finally, terpenes, C13–norisoprenoides and volatile phenols increased their concentration in Albariño cultivar when defoliation was applied to R2.

Effects of shading and bud forcing on vine water relations, yield and grape composition of Macabeo grapevines for "Cava" production

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The cultivation of Macabeo grapevines is of increasing importance in many areas of Spain because its use for production of "Cava" sparkling wines. In the semi-arid and temperate-warm areas of southern Spain, the main challenges faced by grape growers are related with the low must acidity levels when grapes are harvested at the optimum ripening moment for the Cava making purposes. With this in mid, an experiment was carried out in a Macabeo vineyard in Requena (Valencia) to explore the effects of two practices (vine shading and bud forcing after spring pruning) to delay harvest and improve grape composition. Shading was applied with a black shade cloth with a 50% transmittance of the incidence radiation. Shade cloths were installed just after fruit set. Bud forcing was carried out by means of spring pruning applied 20 days after full bloom removing all shoots and leaving two buds per shoot for re-growth. Both practices were successful in achieving the desired grape composition goals, but the harvest delay due to shading was only mild, while bud forcing resulted in a two months delay in the vine harvest time. However, while yield was not affected by vine shading and shading improved vine water status; bud forcing had a very detrimental effect on vine performance, with cumulative carry-over effects on bud fertility. Vine shading is a promising technique for its widespread application, but a cost-benefit analysis should be carried out because of the high initial costs of installation. On the other hand, when applying bud forcing, the rest of cultivation techniques (pest management, irrigation and fertilization and winter pruning) should be adjusted in an attempt to minimize the negative effects that bud forcing might have on yield.

Use of different fermentation temperatures in Cabernet Sauvignon

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The grapes grown in a warm climate could have a low concentration of polyphenolic compounds to obtain wines with color and structure. This work focuses on maximizing in the winery the extraction of all those components of interest for the grape, minimizing as far as possible the coextraction of less interesting or less suitable components for the final wines. The study presents the results of the transfer of polyphenols in the pre-fermentative and fermentative stage over Cabernet Sauvignon musts.

The experiences developed had two phases. The first phase consisted of making a pre-fermentative maceration of the grape at two different temperatures (20ºC or 10ºC) and then fermenting a test at a constant temperature of 22ºC (control) and a second test with a positive temperature gradient from 10ºC at 22 ° C. In the second phase, according to the experience of the first phase, the maceration was done at a single temperature (10 ºC) and the fermentation was done with a shorter temperature gradient over time

The wines resulting from the first phase of the study were wines with little color but very aromatic. The result of the second phase offered wines with more color and more aromatic.

As a conclusion, a standard fermentation temperature gradient can be established to enhance the organoleptic characteristics of the grape, according to the grape variety and its behavior with the temperature in the fermentation.

Keywords: temperature, polyphenolic, red wine, Cabernet Sauvignon

Identification by GC–MS of the main free and glycosidically volatile compounds in grapes from *Vitis vinifera* cv. Albilla over three consecutive vintages

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Galicia (NW Spain) is a traditional viticulture area with several white native grape varieties, however the majority of monovarietal wines are elaborated with three of them: Treixadura, Godello and Albariño. The rest, more than thirty, are employed mixed in plurivarietal elaborations, due to the unknownledgement about their single oenological behaviour. In order to obtain more information about all minor native grape varieties, in EVEGA (Experimental Institute of Viticulture and Oenology from Galicia) are carrying out an investigation project that includes, both ampelographic and analytical parameters. A complete study of these minor cultivars could be useful to elaborate new singular wines contributing to the differentiation of this viticulture area. Searching this differentiation, some producers elaborate singular varietal wines with minor cultivar grapes as Albilla. Conversely, no scientific studies have been published about this variety and its suitability to produce monovarietal quality wines. According to this reason, the aim of the present study is define the volatile composition of Albilla grapes, both in free and bound forms, to identify those compounds or relations between them that allow its varietal aroma characterization. For this purpose grapes from Albilla variety, over three consecutive vintage (2015, 2016 and 2017) were SPME-GC-MS analysed in order to define their aroma profile. The results obtained showed a great number of volatile compounds, mainly in bound form, grouped in 8 chemical families, being the terpene compounds (alcohols, monoterpenes, oxides and diols) the most important group, contributing to the Albilla's aroma with floral (α –terpineol, β-linalol, nerol, isogeraniol, rose oxide) and citrus notes (limonene and citronellol). Benzyl alcohol and 2-phenylethanol in bound form also contributes to increase these floral nuances. The presence of other compounds like, verbenol, β-pinene and isomenthol complete the aroma profile of Albilla variety.

Vineyard zonification based on soil properties and their correlation with must attributes for *Vitis vinifera* (L.) cultivars Brancellao and Caíño Longo

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Assessing the spatial variability of soil properties allows for defining plots with homogenous characteristics within a given vineyard, facilitating site-specific management within the vineyard, adapting irrigation, fertilization, etc. The aim of this study was to define homogenous zones within a vineyard and to correlate soil characteristics with must composition of the grapevine (*Vitis vinífera* L.) cultivars Brancellao and Caíño Longo.

The study was performed in a vineyard from the Designation of Origin Ribeiro (Ourense, NW Spain) in 2017. A 30 x 30 m sampling grid was established and 86 soil samples were collected and analyzed using standard methods for determining soil properties (including texture, pH, ion concentrations). Geostatistics permitted to obtain maps for each of these properties. At harvest, grapes from Brancellao and Caíño Longo were collected in different zones within the vineyard. Must parameters were determined using official methods.

Soil properties showed a great variability; for instance, pH varied between 4.51 and 6.99; clay content from 15 to 40% and Ca concentration between 0.06 and 12.57 cmol kg^{-1}.

For Brancellao, the lower side of the vineyard had higher clay contents and greater concentrations of K and Al than the upper zone. Consequently, musts obtained from vines located on the lower side had higher total acidity and malic acid concentration than those from the upper side.

For Caíño Longo, the lower side of the vineyard had a greater clay conten and K concentration than the upper zone. Musts from plants grown in the lower side had greater total acidity, malic and tartaric acid concentrations and lower pH, polyphenols and anthocyanins than those from the upper side.

In conclusion, two zones were clearly differentiated in the vineyard according to the altitude in which they are located. These zones had different soil properties that influenced must attributes in both cultivars.

Influence of the presence of a cover crop in the agronomic and oenological performance of cv. Chardonnay

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Cover crops are acknowledged to be an interesting tool to produce higher quality grapes in red varieties, as they generally reduce vine vigour and yield. However, their incidence in white wine quality is not that clear, since nitrogen plays an important role, and the cover crops may compete for this nutrient. The aim of this work was to evaluate the long-term effect of a cover crop on grape and wine quality. The study was conducted in a cv. Chardonnay vineyard located in Otazu (Navarra, Spain). During the 10 years prior to the experiment, the vineyard had been managed with a *Festuca arundinacea* and *Lolium rigidum* cover crop. In order to evaluate its incidence, at the beginning of the experiment, part of the rows were tilled and the agronomic and oenological performance of both soil management strategies compared. After 5 years of evaluation, the presence of the cover crop was shown not to affect yield, cluster number or berry weight, but it decreased pruning wood weight and leaf nitrogen content. Regarding grape composition, no differences were observed in terms of sugar content, pH and titratable acidity, but cover-cropped vines produced grapes with lower yeast assimilable nitrogen content. These differences implied a longer fermentation time (21 vs. 16 days), and differences in wine profiles.

Forcing vine regrowth to delay ripening over two months to a cooler period of the growing season

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The most important climate changed-related effects on wine grapes are the advanced harvest times. With increased temperatures and a warmer maturity period, it would be more natural to produce unbalanced wines characterized by high alcohol levels, low acidities, a modified variety aroma and a lack of color. One of the strategies to mitigate these effects is to delay the berry ripening in order that it takes place under a cooler condition (Martínez de Toda *et al*. 2013). For delaying grape ripening, various management techniques have been proposed such as late winter pruning (Zheng *et al*. 2017a), minimal pruning (Zheng *et al*. 2017c) and shoot trimming (Martínez de Toda *et al*. 2014, Zheng *et al*. 2017b). With each of these techniques, ripening can easily be delayed between ten and fifteen days; if we use several of them combined we can delay the maturation between a month and a month and a half.

With the aim of delaying the maturation of the grape by at least two months, the study of a technique consisting of the forced vine regrowth has been studied. Forcing consisted of removing, when developed, the summer laterals and leaves after the growing shoots were trimmed to two nodes. Forcing shifted fruit ripening from more than one month to more than two months, from the warm August to the cooler October and November period. Forced vines produced smaller berries with lower pH and higher contents of acidity and anthocyanins compared to non-forced vines. Forcing is a promising way to restore the anthocyanin to sugar ratio decoupled by the warming climate.

Key words: climate warming, trimming, delayed maturation, anthocyanins, thermal decoupling

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Microbiome assembly in vineyard soils: understanding biodiversity through functional and pathogenic patterns and co-occurrences

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Understanding the biodiversity of agroecosystems is a major aim in a context of sustainable farming. Given the key role soil microorganisms play in crop health and yield, describing microbiome patterns of functional and pathogenic groups in vineyards represent a critical step for a future knowledge-based viticulture. Thus, it is necessary to understand individual and community roles of microorganisms, their relationship with vine health, and the impact of human practices on them.

This work arises with the establishment of a robust database of vineyard soils microbiomes. A total of 700 soil samples from 15 different countries (Argentina, Australia, Chile, Croatia, Cyprus, Denmark, France, Germany, Hungary, Italy, Portugal, South Africa, Spain, India, and USA) has been analyzed by WineSeq technology. The main aim of this work was to establish microbiome patterns in vineyards, defining conserved drivers of biodiversity (core microbiomes), regional/terroir associated features and functional/pathogen co-occurrences networks. For microbiome definition, a 16S/ITS amplicon sequencing approach (Illumina Platform) was applied for bacterial and fungal communities description, respectively.

Ranges of relative abundance for fungal and bacterial pathogens, related with the main vine and grape diseases, have been calculated, also defining geographical-epidemiological patterns. Apart from that, an estimation of the presence and abundance of nutrition-related metabolic pathways (microbial-derived genes related with nutrients (C, N, P, K, S, Fe, etc) metabolism) was analyzed by genome matching through the Tax4Fun routine for the estimation of KEGG-based function predictions. Finally, boundaries of resistance/vulnerability levels of vineyard soil microbiomes has been calculated by a metacommunity definition.

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Use of a seaweed algae extract as biostimulant on Tempranillo blanco grapevines: Effect on grape amino acids content

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Seaweed algae extracts have been used as nutritional supplement or biostimulant in horticulture. These extracts contain several plant growths promoting substances, which have allowed to improve quality and yield of plant crops including grapevines. Additionally, there is low information about the amino acids composition of Tempranillo blanco and the effect of seaweed extracts application on its nitrogen composition is scarce. Due to this, the aim of this work was to study the effect of a commercial seaweed extract, based on *Ascophyllum nodosum* application as biostimulant in two different dosages to Tempranillo blanco grapevines, on grape amino acids composition. The foliar treatments were applied at veraison and a week later, at a concentration of 0.25 and 0.50% v/v. The control treatment consisted in the application of water. Ammonium and amino acids in grapes were analysed by HPLC.

The results showed that the most abundant amino acid in Tempranillo blanco was arginine, followed by proline. Thereby, this grape variety tended to behave as an arginine accumulator variety. The seaweed algae extract treatment applied at the highest dosage to the grapevines induced the synthesis by the plant of most of the studied amino acids, with the exception of glycine, proline, and methionine, compared to the control samples. The grapes from the lowest dosage of seaweed algae extract application presented higher content of aspartic acid, glutamic acid, isoleucine, leucine, and phenylalanine than the ones from control ones.

Consequently, total amino acids concentration in grapes from the treatment with the highest dosage of the seaweed algae extract was higher than in the control samples and in the grapes from the application of the lowest dose of the extract. These results are pioneers in the study of amino acids content in Tempranillo blanco and contribute to understand the seaweed biostimulation effect on grape quality.

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Effect of shifted fruit ripening by forcing vine regrowth in berries development and quality in "Tempranillo" grapevine (*Vitis vinifera* L.) in Extremadura.

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In warmer regions, wine grape ripen their fruits during the hottest portion of the growing season, with negative consequences on qualitative characteristics of grape berries at harvest. The forcing of vine regrowth can be an aggressive but effective technique to delay harvest date (Gu et al. 2012) but, it needs to be evaluated carefully in each growing condition. In an experimental vineyard in Extremadura, forcing was conducted by hedging growing shoots to seven nodes and removing leafs, summer laterals, leaves and primary cluster between 3(F1) – 22(F2) days after anthesis (18th of May and 6th of June). Vines grown under conventional practices were the Control. Vine forcing delayed harvest from 22th of August (Control) to 14th of September (F1) and 19th of october (F2). The shifted of the berry growth period modified the duration of the different fases of the fruit development and reduced the berry size, in higher proportion en F2. The smaller berries had a skin weight ratio similar to the Control, but the proportion of seed weight increased in F2. Quality characteristics of the berry juice were different, and even more those of the wines, highlighting that Control and F1 had similar berry juice content of anthocyanins and total phenolics and smaller than F2; both F1 and F2 differed from the control in the characteristics of the wines, been very high the content of tannins in F2. These preliminary results of a first year of study, indicate the potential of this technique to obtain raw materials with very different characteristics, offering new perspectives to the viticulture of warm areas.

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Application of crown/cyclic proanthocyanidins relative abundances to assess wine authenticity

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Authenticity and origin are main concerns for marketed agri-food products. Traceability tools ensure correct production processes with marketing and economical implications for grape-related products [1]. They are important to define the characteristics of vitivinicultural products. Besides, regulatory practices are required for fair trades [2]. Analytical methodologies have been proposed to assess wine authenticity and origin (e.g. profiling of anthocyanins in red wine) [3]. Proanthocyanidins (PAC) represent a hugely variable oligomeric chemical group. Their diversity of structures may show a correlation with the peculiar wine composition/quality. The PAC composition in wine and grapes has been comprehensively studied. Recent research has identified a new class of proanthocyanidins with a crown/cyclic structure [4,5]. Further efforts were aimed at investigating relations between PAC distribution and technological variables. Accordingly, nineteen wines (nine grape varieties) were investigated. Using statistical approaches, five normalized PAC relative abundance variables (calculated as % proportions of cyclic and - cyclic + non-cyclic - tetramer, pentamer, hexamer procyanidins and tetramer, pentamer prodelphinidins) were applied. The distribution of PAC was strongly influenced by the grape variety, thus the wines obtained with different grape varieties were successfully separated using multivariate statistical analysis. In conclusion, the ratios of crown/cyclic to non-cyclic proanthocyanidins with different oligomer/ring size (4, 5 or 6 terms) are promising chemical markers of the grape blends used in the winemaking process.

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Sensorial and aroma profile of Treixadura wine is improved by sequential inoculation of autochthonous non-*Saccharomyces* and *Saccharomyces* yeasts

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Treixadura is one of the main autochthonous grapevine variety used for production of high quality white wines in Galicia (NW Spain). Some studies have reported the modulation of organoleptic properties of Treixadura wines using different autochthonous strains of *Saccharomyces cerevisiae;* however, data about the potential role of non-*Saccharomyces* yeast are limited. In this work, we have evaluated four indigenous non-*Saccharomyces* yeasts to ferment Treixadura musts. *Lachancea thermotolerans* (Lt93), *Metschnikowia pulcherrima* (Mp278), *Torulaspora delbrueckii* (Td315), *Starmerella bacillaris* (Sb474) were used in sequential fermentations with *S. cerevisiae* (ScXG3). Fermentations were carried out at EVEGA experimental winery in 5L tanks to evaluate their role in aromatic and sensory characteristics of wine. Fermentative aroma analysis by GC/MS-SPME showed that Mp278 wines presented the greatest concentration of esters, acetates and higher alcohols even than the monoculture with ScXG3; in particular, isoamyl acetate, 2-phenylethyl acetate, ethyl hexanoate, ethyl octanoate and hexanoic acid. A statistical correlation was found between analytical parameters, sensory perception and preferences by the tasters. Three groups were clearly differentiated. The best valued wines were those fermented with Mp278 and ScXG3 (floral, intensity, global quality). Wines obtained with Lt93 and spontaneous fermentation were described as fruity. Finally, Td315 and Sb474 wines were poorly valued. These results confirmed that autochthonous yeast strains can be used to modify the aromatic and sensorial profile of Treixadura wines preserving their typicality.

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Vine–shoot chips improve the wine quality

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The current interest for the revaluation of agricultural wastes has demonstrated that vine-shoots, as a result of the vineyard pruning, are an important source of high value compounds, some of them with enological interest. Recently, vine-shoots has been prepared to be used during winemaking, in the same way as oak chips are being used now (Cebrián-Tarancón et al. 2018). In this work, vine-shoot chips with two different sizes from Airén and Cencibel cultivars were added in different winemaking steps to their own wines. Such were: a) before the alcoholic fermentation in both kinds of wines, b) after the alcoholic fermentation in Airén wines and after malolactic fermentation in Cencibel wines, and c) before and after the alcoholic fermentation in Airén wines and before the alcoholic fermentation and before the malolactic fermentation in Cencibel wines. The quality of wines was evaluated at chemical level in terms of colour, volatile and phenolic compounds, and at sensory level by a specific scorecard. Independently of the time in which vine-shoots were added during winemaking, a significant increase of *trans*-resveratrol was observed and a better evaluation was given in the descriptors of the gustatory and olfactory phases.

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Evaluation of glutathione content in white varieties

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Glutathione plays a fundamental role in the oxidative prevention of musts and wines [1]. In the grape, it is mainly in the reduced form, and its content is influenced by many factors (variety, vintage, cultural practices, nitrogen nutrition ...). In musts and wines, it undergoes modifications due to the oxygen exposure, tyrosinase activity, yeast strain ... [2]. In recent years, it has been the subject of numerous studies, since many aspects of its behavior are still unknown.

In this work, the glutathione content was analyzed in grapes, musts and wines of nine white varieties of the D.O. Ca. Rioja (Spain): Chardonnay, Garnacha Blanca, Malvasía, Maturana Blanca, Tempranillo Blanco, Turruntés, Sauvignon Blanc, Verdejo and Viura. Its determination in grapes was carried out with intact berries, which were frozen, crushed and extracted with HCl. The analysis of reduced glutathione (GSH) was performed by HPLC by automatic derivatization with OPA [3].

The obtained results showed important varietal differences in the concentration of GSH. Sauvignon and Tempranillo Blanco presented high contents in grape, must and wine. Verdejo and Turruntés reached high values in grape, which decreased notably in must and wine. In general, the content of GSH decreased in musts due to oxidative processes [2]. In Chardonnay, Garnacha Blanca, Maturana Blanca and Viura increase in wines was observed, which could be caused by the inoculated yeast. The varieties Malvasía and Maturana Blanca presented the lowest level of this compound.

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Early leaf removal in white varieties: impact on the polyphenolic content of wines

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Early partial defoliation is a viticultural practice for yield control in vineyards which consists of removing the basal leaves of the shoots at pre-flowering. The lower availability of carbohydrates limits the fruit set rate and the berry development, which results in smaller, less compact, and less sensitive to cryptogamic diseases clusters. In addition, leaf removal increases the cluster exposure to light and it modifies the composition of the berry, improving the grape juice quality [1]. This technique has been widely studied on red varieties and the obtained results are of great interest [2]. However, leaf removal on white varieties has been less studied.

This work studies the effect of early leaf removal on the polyphenolic content in white wines of Viura and Malvasía varieties. The trial was developed in an experimental vineyard located in Logroño (La Rioja, Spain), during the 2015 vintage.

Results showed important differences concerning the varietal response to this practice, mainly in relation to the phenolic compounds in Malvasia wines. In this sense, the content of the benzoic and cinnamic acids, stilbenes and flavanols decreased, whereas flavanols increased significantly in Malvasia wines due to the early leaf removal. On the contrary, only the stilbene content in wine decreased for Viura variety. The effect of leaf removal on polyphenolic compounds is related to the improvement of the sanitary status of the grape and to the lower compactness of the bunch [2]. The increase of flavonols content observed in Malvasia wines is in agreement with those showed by other authors for red varieties.

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Effect of carbonic maceration and pectolytic enzymes on the polyphenolic composition and foam parameters of red base and sparkling wines

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Red sparkling wines constitute an attractive product for the consumers. The main problem to produce them is to obtain a base wine with the adequate characteristics, i.e., moderate alcohol content and good color intensity and mouthfeel. Phenolic compounds are considered quality parameters because they are related to the wine organoleptic characteristics, mainly color, bitterness and astringency. In addition, polyphenols are related with foamability, an important parameter for the quality of sparkling wines. Therefore, the aim of this work was to evaluate the effect of two different winemaking techniques on the polyphenolic composition of red base and sparkling wines: (i) carbonic maceration (PM-CM); (ii) traditional winemaking with addition of pectolytic enzymes (PM-E). Two control wines were also made by traditional red winemaking with ripe (M-C) and pre-mature grapes (PM-C). PM-E and PM-CM red base wines showed significant lower contents of total anthocyanins, proanthocyanidins and flavonols than the control red base wines. M-C red base wine showed the highest concentration of esterified acids followed by PM-CM while PM-E and PM-C red base wines showed a significant lower concentration. Ageing on yeast lees produced a generalized decrease in all phenolic compounds and the differences showed on red base wines were maintained. Foam parameters were measured by Mosalux method [1]. The values of HM (foamability) and HS (persistence of foam) measured on red sparkling wines were in agreement with those described for white and rosè sparkling wines [2].

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Effect of different types of stopper on the phenolic, volatile and sensory characteristics of four wines from South Tyrol (Italy)

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Since the 18° century, cork has been one of the most popular materials used to close the wine bottles. However, cork stoppers have some weaknesses, due to the heterogeneity of the cork structure. Their production is difficult to standardize: low quality corks may cause the diffusion of excessive gas amounts because the oxygen transmission rate (OTR) in cork stopper is highly variable [1]; in addition, cork taint is one of the most serious defects to the wine [2]. These issues have led to the development of caps made of alternative materials, less expensive and with standard characteristics [3]. The technologies applied to the production of alternative stoppers allow controlling the range of OTR.

In this work, four red or rosè wines from South Tyrol (Italy) were closed with two different selected stoppers: a conventional one and a new typology of a sanitized cork micro-granules blend of natural cork and polymers without addition of glue. Merlot, Lagrein, Lagrein rosè and St. Magdalener were analysed over 6 months of bottle-aging to evaluate their effect on the volatile composition, sensorial evolution and phenolic profile. The statistically significant differences between the wines closed with the two types of stopper were discussed and the crucial influence of the type of stopper on the chemical and organoleptic wine characteristics are described. This work highlights the importance of choosing the suitable typology of stopper to preserve the quality attributes of each type of wine.

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Changes in wine aroma influenced by ultrasonic bath treatment

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High power ultrasound is often regarded as potential alternative method for improving the wine quality, especially for shortening the wine aging process through mechanisms influenced by formation of acoustic cavitation of microbubbles. The ultrasound processing is usually carried out by the use of ultrasonic probe device or ultrasonic bath, wherein the ultrasonic bath is especially emphasized as less invasive ultrasonic technique. The aim of this research was to investigate the influence of ultrasonic bath treatment on the aroma compounds of white wine Graševina (Vitis vinifera L.). During the treatment, different process parameters were applied: amplitude (100, 60 and 40%), frequency (80 and 37 kHz), bath temperature (20, 40 and 60 °C) and duration of treatment (20, 50, 65 and 90 min). Immediately after the treatment, aroma compounds were determined and compared to control, non-treated wine. The solid-phase microextraction and gas chromatography coupled with mass spectrometry (SPME-GC/ at MS) was used for aroma compounds analyses. Results showed different trends regarding the applied sonication parameters. The most important discriminant factors affecting the changes in aroma composition were temperature and treatment duration, wherein higher temperatures as well as longer treatment durations resulted in significant decrease of analyzed compounds. Regarding the applied amplitude, changes were pronounced in a much lesser extent than those of temperature and duration. Finally, no clear conclusion could be drawn regarding the variations in frequency level. These results suggest that application of ultrasonic bath systems for wine treatments should be performed at lower bath temperatures, as well as shorter treatment durations.

Effect of crop level in different water regimes on Verdejo wine volatiles

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The volatile composition responsible for wine aroma depends on several factors, including variety, degree of ripeness, climate, vineyard management and winemaking aging practices. Water management is a fundamental tool for controlling reproductive growth and grape quality. Also, the overall effect of irrigation might produce changes according to other cultural practices, particularly those associated with crop level.

The present study aimed to determine the effect of crop level, modified through cluster thinning, in different water regimes on the volatile composition of Verdejo wine. This work was conducted from 2012 to 2014 seasons in an experimental vineyard of *Vitis vinifera* cv. Verdejo located in Valladolid (Spain), D.O. Rueda.

The cluster thinning was applied at the beginning of veraison, with a reduction of the third part of clusters (A) and compared with a control treatment (T). T and A were applied on different water regime treatments: rainfed (R0), drip irrigated at 25% ETo (R25) and 50% ETo (R50). Drip irrigation was applied weekly from the end of vegetative main shoot growth until harvest. The grapes from each experimental treatment replication were vinified in duplicate at the University of Valladolid (Palencia, Spain), following a classical pattern of development of white wine. Wine volatile compounds were identified and quantified by GC-MS according to Oliveira et al. (2006).

The results showed that alcohols, ethyl esters and volatile acids were the most abundant volatile families in Verdejo wine. Cluster thinning treatment (A) applied in rainfed (R0) induced an increase of total volatile concentration in 2012 and 2013 vintages, whereas these compounds were not affected substantially in 2014. Alcohols, ethyl esters and lactones were the most affected volatiles increasing their concentration in R0-A. A trend to increase the other volatiles groups quantified (volatile acids, C_6-compounds and phenol volatiles) in R0-A was also observed in the driest year (2012).

Cooperage and year variability in volatile composition of oak wood toasted during barrel production and red wines

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It is well known that oak barrel aging of wines improves theirs quality since favours wine stability and the extraction of desirable oak aromas. The barrel toasting process is probably the most important factor that influence on the chemical composition of the wood during barrel production. This process produces a thermal degradation of wood components by pyrolysis and hydrothermolysis, increasing the amount of several volatile compounds [1-2]. Each cooperage has their own process that has to be well controlled in order to maintain the quality of their barrels. In spite of this, high variations in volatile composition of toasted wood can be found even within the same batch of barrel manufacture.

The aim of this work was to determine the volatile composition of oak woods used in barrel production for wine aging from different cooperages in four consecutive years, and their effect on volatile composition of red wines.

Fifteen oak wood samples (French and American) from nine cooperages were studied. The toasting degree of all woods was medium plus. Wood volatile compounds [3] and wine volatile compounds [4] were determined. The final organic fractions were analysed by GC-MS.

Great differences were found in volatile compounds of toasted wood among cooperages especially in phenyl ketones (70%) and volatile phenols (50%). Whiskeylactones and phenyl ketones showed the highest variability by years within the same cooperage.

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Influence of vineyard Normalized Difference Vegetation Index obtained from satellite images on Verdejo wine characteristics

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The use of satellite images or drones for precision viticulture is increasingly widespread. One of the indexes that can be used is the Normalized Difference Vegetation Index (NDVI), as a first step to establish differentiated management units, since it is well known that the vine vegetation development is related to wine quality [1-2].

The aim of this work was to determine the influence of vine vigor index on the white wine characteristics. Three vineyards of Verdejo grape variety with different Normalized Difference Vegetation Index (NDVI) obtained from satellite images (Sentinel 2) were selected.

Verdejo wines from the three different vineyards selected by their different vigor (high, medium, low) were elaborated following the traditional white winemaking. The enological parameters [3], total and individual phenolic compounds [4] and volatile composition [5] was determined.

Wines elaborated from grapes of low vigor vineyard showed the lowest alcohol content and the highest dry extract. Statistically significant differences were observed in hydroxycinnamic acids, as caffeic and caftaric acids. Respect to volatile compounds, the Verdejo wines elaborated from grapes of low vigor vineyard showed lower ethyl esters of fatty acids but a much higher content of alcohol acetates, compounds associated to fruity notes.

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Effect of polyphenol extracts of *Vitis vinifera* skins and seeds on the growth of antibiotic multiresistant *Escherichia coli* strains

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Antibiotic overuse has led to the rise of multidrug-resistant bacteria and global dissemination of antibiotic resistance (AR). *Escherichia coli* is considered to be an excellent indicator of AR because it is part of the normal microbiota of the gastrointestinal tract and it also occurs in the environment. Extended spectrum beta-lactamases (ESBLs) is a genotype that confers resistance to most β-lactam antibiotics. On the other side, polyphenols have been reported to possess a remarkable antioxidant activity as well as potential antibacterial activity, and grape pomace may become a suitable candidate to combat AR dissemination. The aim of this work was to study the effect of polyphenols extracted from red grape skins and seeds on the growth of antibiotic multiresistant *E. coli* strains.

Six ESBL resistant *E. coli* strains and susceptible control strains were studied. Grape polyphenol extracts were obtained from red grape skins of *Vitis vinifera* L. cv. Tempranillo (TG) and Graciano (GG) and from seeds (TS and GS). Antimicrobial activities of the extracts were determined in absence and in presence of a subinhibitory concentration of ampicillin. Results showed that all the strains were inhibited by the extracts and their effect was bacteriostatic as bacterial growth was recovered when the extracts were removed. Minimal inhibitory concentrations that inhibited the growth of 50 % of the tested strains were 6.25 mg/ml for GG and TG, and 1 mg/ml for TS and GS. Total anthocyanin (402.5 mg/g) and flavonol (162.9 mg/g) contents of GG were higher than the respective contents of TG (246.2 and 87.9 mg/g). Both TS and GS showed similar contents of total flavanols and hydroxybenzoic acids (747.8 ± 22.1 and 7.65 ± 0.2 mg/g respectively). Results showed in all cases that the extracts inhibited the growth of the multiresistant strains and potentiated the effect of the antibiotic subinhibitory concentration.

Ultrasound assisted extraction of anthocyanins and total phenolic compounds in wine lees.

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Wine lees are a by-product that is generated after the fermentation of the red and white grapes musts. These lees have a variable composition and are mainly made up of yeasts, tartaric acid, colloids, polyphenols and ashes content (Pérez-Serradilla & Luque de Castro, 2008). Ultrasound Assisted Extraction has been used to extract both anthocyanins and total phenolic compounds. The optimization of the extraction variables (% methanol, pH, temperature, amplitude, cycle and ratio mass/solvent) was performed using the Box-Behnken methodology considering as independent variables the total amount of anthocyanins on the one hand and total phenolic compounds on the other hand. Fifteen major anthocyanins were identified (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside, delphinidin-3-O-(6”-acetyl)-glucoside, cyanidin-3-O-(6”-acetyl)-glucoside, petunidin-3-O-(6”-acetyl)-glucoside, peonidin-3-O-(6”-acetyl)-glucoside, malvidin-3-O-(6”-acetyl)-glucoside,cyanidin-3-O-(6”-p-coumaroyl)-glucoside, petunidin-3-O-(6”-p-coumaroyl)-glucoside, malvidin-3-O-(6”-cis-p-coumaroyl)-glucoside, peonidin-3-O-(6”-p-coumaroyl)-glucoside and malvidin-3-O-(6”-trans-p-coumaroyl)-glucoside) using UHPLC-Q-ToF-MS. Anthocyanins were analyzed by UHPLC-UV-Vis and total phenolics by the Folin-Ciocalteu spectrophotometric method. The percentage of methanol and the temperature of extraction are the most critical variables in extracting anthocyanins and the percentage of methanol and the cycle of extraction are the most critical for the total phenolics. The extraction kinetic was also studied using the best extraction conditions. Five minutes of extraction was sufficient to extract the maximum amount of both total phenolic compounds and total anthocyanins. The methods developed have a good repeatability and reproducibility (RSD < 5%). The two methods developed have been used for the analysis of anthocyanins and total phenolics in different red wine lees collected in cellars of the province of Cadiz (South of Spain).

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Improvement of Tempranillo grape and wine phenolic composition by the foliar application of methyl jasmonate: a three–vintage study

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Phenolic compounds include a heterogeneous group of secondary metabolites that play diverse biological functions, including plant protection against stress factors, such as UV radiation or pathogen incidence. These compounds also contribute to grape and wine health-promoting and organoleptic properties, as they are responsible for the wine colour, mouthfeel properties, or ageing capacity. Recently, the exogenous application of elicitors, which are molecules able to trigger plant defence mechanisms, has been described to improve grape phenolic composition.

The aim of this study was to evaluate the effect of the application of the elicitor methyl jasmonate on the phenolic composition of Tempranillo grape and wine during three consecutive vintages (2014, 2015, and 2016). The vineyard was located in Alfaro (La Rioja, Spain). Methyl jasmonate was foliar applied at the beginning of veraison and one week later and compared with an untreated control. Grapes were vinified and the grape and wine phenolic composition was determined by SPE-HPLC/DAD.

The results showed that the treatment increased the grape total anthocyanins and stilbenes in 2015 and 2016 seasons. Moreover, grape total flavonols were increased in 2016. On the contrary, no significant differences were found regarding grape flavanols and hydroxycinnamic acids. In wine, the application of methyl jasmonate only led to significant differences in total anthocyanins content in 2014. Moreover, the elicitor treatment improved wine color intensity and ionization index in two of the three years of the study.

Overall, methyl jasmonate application could be an interesting tool for improving grape anthocyanin and stilbene composition as well as wine chromatic parameters.

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Chemical breakdown of the lignocellulosic residue from a bioestimulant vine–shoot extract

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Previous research have demonstrated that vine-shoot aqueous extracts have an interesting composition (Sánchez-Gómez et al., 2014) and also, they could act as a vineyard biostimulant (Sánchez-Gómez et al., 2016, 2017). To improve the composition of such extracts, it may be interesting to carry out an exhaustive chemical hydrolysis of the solid residue resulting from the previous aqueous extraction, with the aim to facilitate the liberation of volatile compounds still present in the lignocellulosic fraction. To achieve this aim, vine-shoots from Cencibel variety previously extracted by conventional solid liquid extraction with water (Sánchez-Gómez et al., 2014) were submitted to a second extraction with two different NaOH aqueous solution by means of microwave procedure considering two extraction temperature. Each combination of NaOH solution and microwave temperature was extracted five times using the same proportion solid residue. Among the results obtain, it is worth highlighting those obtained for guaiacol and eugenol compounds, which showed a higher recovery when the most concentrated NaOH aqueous solution and higher temperature was used. Also, in both cases, with three extractions was possible to obtain the 90% of the total of such compounds still present in the lignocellulosic fraction. The concentration of both compounds supposed an increment of more than 12 times in guaiacol and 30 times in eugenol respect to the initial vine-shoot aqueous extract.

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Effect of post-harvest CO_2 atmosphere saturation grape treatments on cv. Cabernet Sauvignon red wines

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The aim of this work is to study the chemical and sensory changes on Cabernet Sauvignon wines produced by grapes treated with CO_2 atmosphere saturation. The post-harvest grape treatments were performed at two different temperatures (T1 = 0ºC) and (T2 = 12ºC) during 15 days in saturated carbon dioxide atmosphere conditions. The phenolic composition and the aromatic profile of wines were evaluated at three different times, just after the malolactic fermentation (MLF), three months later, and as bottled wine. Some differences on the chemical and sensory profile of the studied wines were found. Results showed that, in general, T1 wines contained high level of anthocyanins than control and T2. Regarding the aromatic profile of wines, it has been also observed that T1 wines obtained a higher concentration of ethyl esters, at three months after the MLF. Furthermore, it should also be noted that T1 wines maintained a greater quantity of fresh aromas (roses, pollen, yogurt or perfume), especially related to the content on isoamyl alcohol and 2-phenylethyl alcohol. In general, T1 wines seem to show the most aromatic expression after bottled. From a sensory point of view, T1 wines showed higher freshness and unctuosity and lesser astringency and greenness. T1 wines were generally distinguished and preferred by tasters. This study opens up new winemaking strategies adapted to the climate change in order to produce different style of wines, fresher and less astringent. For future experiments, it would also be interesting to evaluate factors, such as varieties, time of harvest and maceration.

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Influence of different types of vineyard pruning on the volatile profiles of several grape varieties

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Grape aromatic characteristics are very important for the production of quality wines. The concentrations of volatile compounds and precursors in grape berries are highly influenced by viticultural practices [1]. For this reason, the aim of this work was to evaluate the influence of different vineyard pruning methods on the volatile profiles of grapes. To this end, volatile profiles of three grape varieties (Tintilla de Rota, Tempranillo and Shyraz) obtained from vines with three pruning methods (double guyot as control, triple guyot and smart) over two harvests (2016 and 2017) were determined. The grape samples came from Jerez de la Frontera (Andalusia, Spain), a warm climate zone. Must volatile compounds were determined by sequential sorptive extraction with Twisters by immersion (SBSE) and headspace (HSSE), followed by GC-MS analysis [2]. A total of 160 compounds were determined. As expected, the three varieties presented different volatile profiles, the amount of aldehyde stood out in the Shyraz variety, and compounds such as isoamyl acetate, methyl heptanoate, methyl nonanoate, 4-terpineol or 2-decen-1-ol were not detected in the Tintilla de Rota variety. The lowest total values of volatile compounds were obtained in the case of "triple guyot" pruning and smart pruning produced the highest total values of acetates for the three grape varieties studied. However, in most cases, the different pruning methods changed the volatile profile of each variety differently.

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Study of the oxygen consumption rate of different wine polyphenol fractions

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Micro-oxygenation (MOX) is a technique widespread used in wineries with the purpose of bringing about a positive impact on wine quality, in terms of colour, aroma and texture. It is known that wines show marked differences in the ability to consume oxygen, which mainly depend on their polyphenolic composition. Among these compounds, Aldehyde Reactive Polyphenols (ARPs) seem to play a relevant role, being ARPs content determinant both in the oxygen initial consumption rate and in the acetaldehyde accumulation (1, 2).

The aim of this work was to investigate the impact of MOX (30 mg/L/month) in different wine polyphenolic fractions obtained by means of gel permeation chromatography and solid phase extraction in comparison with the same fractions kept in anoxia. For it, a young red wine with high TPI was selected. Freeze-dried fractions containing separately anthocyanins, monomer flavonols and flavanols and polymeric tannins and also different combinations of them, including freeze-dried wine itself, were dissolved in a wine model solution and studied in terms of oxygen consumption and sensory and chemical changes. MOX experiment was performed during 37 days followed by a period of 6 weeks inside an anoxic chamber. Dissolved oxygen was measured using a non-invasive method based on luminescence.

The results showed significant differences in the oxygen consumption rate due to the chemical composition of the different fractions studied. Six significant different clusters were observed attending to their different behaviour against oxygen. Chemical and sensory changes were noticed due to the dose of oxygen applied.

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Discrimination of aging wines with alternative oak products and micro-oxygenation by FTIR-ATR

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The use of alternative oak wood products (AOP), such as chips, cubes and staves, among other, from different geographical origins is a common practice for wine aging, where the micro-oxygenation (MOX, adding small doses of oxygen constantly over time) is essential to obtain a final wine more stable in time and with the same characteristics of barrel-aged wine. The aim of this work was to identify if with spectroscopic techniques it was possible to discriminate wines after 10 years in the bottle and aged with alternative oak products (chips and staves), from different oak woods (American, French and Spanish) and a floating micro-oxygenation (20 ppb), compared to those aged in barrels. The spectral information and analysis were performed in a FTIR-ATR, with 128 scans per spectrum at a spectral resolution of 8 cm^{-1} in the wavenumber range from 4000 to 450 cm^{-1}. Principal component analyses of spectral information were performed using the Unscrambler® X. The results indicate that with this technique it is possible to clearly separate the wines aged by the three systems (chips, staves and barrels) in the case of American oak. In the case of French oak, wines aged in chips were clearly differentiated from wines aged in staves by presenting those aged in intermediate barrels. It is also possible to clearly separate aged wines with different Spanish oak systems. The application of FTIR-ATR appears to be a powerful technique for discriminating the quality of wines aged by different AOPs and wood barrels from different geographical origins.

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Wine aging in barrels with different oxygen transfer rate

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Wine aging is a long and complex process in which wine improves its chemical and sensory properties. The entry of oxygen from the air into the barrel plays an essential role in this process, and depends on the type of barrel, the origin of the wood, the treatments carried out in the barrel, etc. This paper presents the results of the ageing of the same wine in barrels with different oxygen transfer rates (OTR). For this purpose, the French oak wood staves were classified according to its OTR and barrels were built with different OTR. The results of the evolution of the same red wine during 6 months in 10 barrels with different OTR are presented. The evolution of the phenolic and colour compounds has been evaluated, as well as the compounds released by the oak wood in the same wine aged in those 10 barrels. The results indicate that it is possible to make barrels with different OTR and that the greater or lesser amount of oxygen that the wine receives affects its evolution in the barrel. It has been found wines showing different characteristics in terms of the compounds released by the wood, phenolic compounds and colour according to total oxygen received.

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Relevance of mannoprotein and flavanol structure on the strength and the forces driving their interaction with salivary proteins

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Astringency is an important organoleptic property of red wines that mainly results from the interaction between phenolic compounds and salivary proteins [1]. This sensory perception in red wine can be modulated by mannoproteins (MPs), since they are described to interact with the phenolic compounds, thus affecting the salivary protein-phenolic compound aggregation [2]. Wine MPs, which can be excreted by yeast during fermentation or released by cell walls break down (yeast autolysis), are glycoproteins from yeasts consisting mainly of mannose but also glucose connected to the cell matrix (β-1,3 glucan) through covalent bonds. [3]. Consequently, the structure of MPs can present several differences. Moreover, protein-flavanol interaction can take place mainly through hydrophobic interactions and hydrogen bonds so the structural features of both, flavanols and MPs, could affect their interaction.

The objectives of this work were: i) to assess the possible relationship between the structural features of MPs and their ability to interact with tannins and to affect the tannin-salivary protein interactions, ii) to characterize the main mechanisms and the forces driving those interactions. The structural features of different MPs commercially available have been characterized and their interaction with flavanols and with flavanols/salivary proteins mixtures has been assessed by means of HPLC-DAD-MS. Moreover, the interaction between flavanols and mannoproteins and/or salivary proteins has been studied by using ITC. Results pointed out that both the tannin and mannoprotein structural features determined the strength of the interactions and, therefore, the capability of MPs to affect flavanol-salivary protein aggregation. Moreover, the analysis of thermodynamics parameters obtained by ITC has allowed the differentiation of the possible mechanisms and the driving forces involved in these interactions. The results pointed out that the ratio manose:glucose and the protein percentage in the mannoprotein structure may influence the mechanism by which the MPs affect the flavanol-salivary protein interaction.

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Characterization of phenolic composition in Carignan wines from the Maule Valley (Chile)

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Carignan vineyards located in the Maule Valley (Chile) have been rediscovered by famous viticulturist and winemakers, producing high quality wines which have been recognized worldwide. Phenolic composition plays a key role on the sensory attributes of wines, giving, a better color, bitterness and flavour. However, several factors affect grape and wine phenolic composition, such as grape variety, soil and climate conditions, viticultural practices, oenological procedures, and aging conditions.

Thereby, the aim of this work was to characterize wine phenolic composition from Carignan (*Vitis vinifera* L.) grapevines ungrafted and grafted with País (*Vitis vinifera* L.), from ten different bioclimatic zones of the Maule Valley (Chile), growing in rainfed conditions. Phenolic compounds (anthocyanins, flavonols, flavanols, hydroxycinnamic acids and stilbenes), were analyzed by HPLC-DAD.

The results showed that total anthocyanins concentration in Carignan wines ranged from 68.26 to 309.23 mg/L (Tru and Cur, respectively), being the most abundant phenolic compounds. Total flavanols content in wines varied from 28.90 to 138.55 mg/L (Hdm and Cur, respectively). Total flavanols concentration in wines ranged from 27.52 to 72.12 mg/L (Tru and Cur, respectively). Total hydroxycinnamic acids content in wines varied from 23.84 to 51.39 mg/L (Cdn and Sau, respectively). *trans*-Piceid were the only stilbene identified, and its concentration in Carignan wines varied from 0.18 to 0.62 mg/L (Mel and Cur, respectively). In general, high contents of phenolic compounds were obtained in wines from the warmer areas of the Maule Valley, while low concentrations of these compounds were found in wines from cooler sites. In accordance with the multivariate analysis, the composition of phenolic compounds of the Carignan wines was determined by the weight of 100 berries, which was conditioned by the location. País rootstock barely affected the wine phenolic composition.

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After NitroPortugal – the implementation of Nitrogen–footprint concept at the farm level in NEP

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Nitrogen (N) is a key nutrient, indispensable for all living organisms including Man. For over one century, demographic increase and agriculture intensification allowed to feed the world population but came with high costs. N has the most altered cycle, and constitutes the most pressing environmental issue faced today. Despite the the available scientific, technical or practical knowledge worldwide, it has not been fully utilized and a holistic approach is needed to solve the problem. NitroPortugal addresses how to improve the scientific output of Portugal, at the same time strengthening the potential for N policy implementation developing around the whole N-cycle WAGES concept (Water, Air, Greenhouse-gases, Ecosystems/biodiversity and Soil) launched by the European Nitrogen Assessment, under the consensus that N is an emerging issue, with impacts in all the environmental compartments, and both human health and social implications. Despite the well known negative effects and the long term research on N pollution, the N story is not yet widely known by the general public. A N-footprint concept was created to comunicate the importance and effects of N, with the creation of a first personal N-Calculator tool to show how personal consumption choices impact N pollution. To go further in raising public awareness about the pros and cons of N use in agriculture and related impacts in the environment, a N-footprint for the production aims at helping the farmers to reduce their product's N-footprints while maintaining yield and quality. The NEP Operational Group was created to produce marketable wine grapes and processing tomato, with low N-footprint, through differentiated management of fertilizers and irrigation.

Identification and distribution of 3–methyl–2,4–nonanedione precursors in grapes and wines. How can they help us to predict the aging potential of red wines

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The aromatic composition of wine evolves through exposure to oxygen during aging. Although this development is usually positive in red wines, it can be detrimental in certain wines, resulting in a rapid loss of fresh, fruity flavors. Prematurely aged wines are marked by intense prune and fig aromatic nuances that dominate the desirable bouquet that can be achieved through aging. This aromatic fault is, in part, caused by the presence of 3-methyl-2,4-nonanedione (MND) in concentrations above its detection threshold [1,2]. Thus, high levels of MND can negatively impact wine quality and aging potential. Determination of the precursors of MND and the mechanisms by which MND is formed and evolves in wine will improve our ability to understand and predict wine stability during aging.

Previously identified as precursors to MND in soybean oil [3], furanoid fatty acids (FFA) were measured in grapes and wines by GC-MS/MS. We demonstrated that its oxidation yielded MND in model wine and red wine. We also report the identification in wine of 2-hydroxy-3-methyl-4-nonanone, reminiscent of anise and plastic-like corresponding to the reduced form of MND. This compound results in the reduction of MND during alcoholic fermentation as well as hydrolyse from glycosylated precusors. The chemical synthesis of the two diastereoisomers allowed us to evaluated for their impact on MND formation in wine. Additionally, the oxidation of MND was found to yield 3-hydroxy-3-methyl-2,4-nonanedione (creamy, plastic-like), identified for the first time in red wines. We quantified by SPME-GC-MS/CI these three compounds in more than 80 Merlot and Cabernet Sauvignon wines from California, Bordeaux, and Switzerland. Their impact on wine aroma and detection thresholds have been evaluated. Gaining insight into the formation and evolution of MND and other significant aroma compounds will enable more accurate predictions of the oxidative behavior and aging potential of wines.

Acknowledgements Région Aquitaine

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Investigating the impact of extended maceration on the grape berry cell wall breakdown and the release of phenolic compounds.

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Wine chemistry is influences the wine's consumer's acceptance. The use of different winemaking techniques, such as extended maceration, seeks to alter and control the extractability of specific phenolic compounds in red wines. Although a prolonged skin contact time is meant to favour an increase in wine tannin concentration (Casassa, 2017), the diffusion of certain cell wall components (Guadalupe & Ayestarán, 2007) may interact with certain phenolic compounds and thereby alter the phenolic stability (Poncet-Legrand *et al.*, 2007). The present study evaluated the impact of three weeks of extended maceration after fermentation on the phenolic release into the wine as well as on the cell wall polysaccharide deconstruction in Shiraz wines. The evolution of the polysaccharides in the grape pomace were evaluated with the use of the Comprehensive Microarray Polymer Profiling (CoMPP), whereas the wine phenolics in the fermenting must were measured with spectrophotometric methods. The results showed an impact of the extended maceration on the polysaccharides evolution, but also found that a longer skin contact period not always favour the increase of wine total phenolics (TP) and tannins.

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Impact of the use of different inert gases and cork closures on the chemical and sensory profile of white wines

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The rapid evolution of white wines in bottle is a concern for enologists, and ensuring an adequate quality for as long as possible is crucial for wineries. In this study, the use of different inert gases during bottling and cork closures were evaluated in order to preserve the quality parameters of young white wines. The study was carried out from Verdejo and Sauvignon Blanc grapes. Three different gases, carbon dioxide (CO_2), nitrogen (N_2) and argon (Ar), were used for the inertization of bottles during the bottling process. In addition, six types of cork closures with different characteristics and O_2 permeability were evaluated. Wines were analyzed at four different times, at bottling and after 3, 9 and 12 months of storage. The phenolic profile, color parameters, aroma compounds and sensory analysis were evaluated. The results showed sligth differences on the color evolution, however different preservation of the fermentative aromas were obtained during the experiments, regarding gases and corks. Ethyl butyrate (pineapple), ethyl octanoate (flowers), isoamyl alcohol (platan), 2-phenylacetate (roses and honey) and 2-phenyl-alcohol (fruit) were the most affected aroma compounds by the treatments. In addition an increase of some oxidative aromas of wines were found during the period of storage, especially Strecker aldehydes and Alkenales.

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Targeted and untargeted ^{1}H–NMR analysis to investigate Bordeaux wine quality and authenticity

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Traceability of wine is today a consumer demand and a scientific challenge. The methods of analysis must be able to control three fundamental parameters: the geographical origin, the grape varieties, and the vintage. With these focus, the Bordeaux Wine Council (CIVB) supports the creation of a VRAI platform (Wine-Research-Authenticity-Identity) within the ISVV (Institute of Vine and Wine Sciences). This platform aims to develop analytical tools to guarantee the origin of a wine.

Quantitative Nuclear Magnetic Resonance (qNMR) may be a great tool to help authenticate wines. The acquisition of a large number of wine parameters requires a small volume (a few hundred microliters) and the analysis is performed in a few minutes. This innovative analytical technique can therefore be useful to characterize wines quality and authenticity particularly in the context of priceless wine.

Today, the absolute quantification of many molecules has been determined. Following these quantifications, it was possible to detect cases of fraud by comparison with the original wine provided by the castle. Wines authentication seems also possible with the help of a database regrouping wines from different geographic origins, vintages, and grape varieties. These first results highlight the discrimination of wines according to their origins and grape varieties.

The effect of pH and organic acids on anthocyanins and pigment stability of red wine

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Red wine aging is an oxidative process in which grape pigments, the anthocyanins, undergo a great number of chemical reactions origining the long-lasting color of red wine. Anthocyanins are extracted from grape skins during maceration/fermentation but part of them precipitate on grape pomace and/or on the surface of winery tanks during winemaking and wine aging. Wine is even an acid solution containing several organic acids and each of them could differently affect anthocyanins extraction and stability. Although the effect of pH on anthocyanins equilibria (Brouillard and Delaporte, 1977) and copigmentation (Boulton, 2001) is well known, few reports on anthocyanins solubility as function of pH, as well as on the role of the nature of organic acids in preserving pigments degradation during oxidative aging are reported. With the aim of better understand the role of pH and organic acids on anthocyanins stability two experiments were performed. First, the effect of pH (ranging from 2.8 to 4.0) on malvidin-3-monoglucoside solubility and on the copigmentation was investigated by NMR and spectrophotometric techniques, then the effect of the main grape organic acids (tartaric, malic and citric) during degradative oxidation of malvidin-3-monoglucoside was investigated by NMR, HPLC and spectrophotometry. Experiments were first performed in model solutions and then in real wine. Results showed that increasing the pH a decrease of solubility was detected. At pH 3.3 a drop of solubility and copigmentation (four time less) occurred. Concerning organic acids data showed that tartaric acid preserves native anthocyanins from oxidative degradation more than malic and citric acid. These data have important practical implication for the management of pH and acidity during winemaking.

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Chemical analysis and sensory assessment of flavour compounds in Furmint wines

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The hungarian Furmint variety is a so called "neutral" grape variety, which means that the concentration of terpene compounds is low, thus the final aroma sensation depends on the concentration ratio of many compounds. Consequently, in the absence of a dominant perfuming note, Furmint may express the terroir better. In orded to examine this effect on a selected variety, we must have prior information about the typical sensory and chemical composition of the selected wine. Nevertheless, there is no information about it in the scientific literature, so initially we have to determine the characteristic sensory and chemical composition of Frumint wines. Our study provided a first insight into the sensory attributes and volatile composition of the single varietal dry Furmint wines. An HS-SPME-GCMS method were used to analyse 30 commercial Furmint wines from different regions of Hungary, and 4 experimental wines from the same vinyard. 131 compounds were identified by mass spectral databases (NIST07 and Wiley's Flavour and Fragrance DB) and analytical standards. The headspace was mainly constituted by esters (40) followed by alcohols (20), phenolic compounds (14), terpenes (13), organic/fatty acids (10), furan derivatives (7), and others (23). After that, a targeted method was developed for these 131 compounds, which were used to analyse wines from three different slope. Some compounds show a significant difference between slopes.

Furthermore, a descriptive analysis investigated the sensory attributes of the commercial Furmints by 10 panelists. Wines were descripted with terms citrus, minerality, apple, honey, pineapple, banana, yeast etc., in order of the number of perceptions.

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Schizosaccharomyces pombe and *Lachancea thermotolerans* as an alternative to malolactic fermentation

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The classical way to make red wine is based on the use of *Saccharomyces cerevisiae* yeasts during alcoholic fermentation and *Oenococus oeni* bacteria during malolactic fermentation. This traditional winemaking methodology produces commercial stable red wines from a microbiological point of view. This work explains the use of a new red winemaking biotechnology that uses the combination of *Lachancea thermotolerans* and *Schizosaccharomyces pombe* yeasts as an alternative to the conventional alcoholic and malolactic fermentations. *S. pombe* consumes malic acid while *L. thermotolerans* produces lactic acid in order to avoid an unnecessary deacidification in low acidic musts from warm viticulture areas such as the south of Spain. This methodology also reduces some malolactic fermentation hazards for human health such as biogenic amines and ethyl carbamate while it improves the colour and aroma profile.

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Impact of *Botrytis* on the characteristics and foaming properties of yeast macromolecules released during a model juice fermentation

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Botrytis cinerea is an important fungal pathogen particularly dreaded in cool climate vineyards. It is responsible for important damage, including the decrease in foamability of sparkling wines, such as Champagne. Proteins are largely involved in the stabilization of Champagne foam despite their low concentration. Other works demonstrated changes in must proteins originating from botrytized grapes. Nevertheless, we have very few information concerning the effect of *Botrytis* contamination on the biochemical and physico-chemical characteristics of proteins released by *Saccharomyces* during fermentation. To answer this, the proteolytic activity of a *Botrytis* supernatant was compared with that of a Chardonnay grape juice produced with bunches contaminated by *Botrytis* (10%, visual estimation). A synthetic must was inoculated with an enological yeast (strain 18-2007, used as a reference) and with strains presenting or not a cell wall mutation with or without 1% of *Botrytis* culture supernatant. Macromolecule fractions of the 6 wines obtained after alcoholic fermentations were purified and analyzed by SEC-MALLS, and SDS-PAGE (AgNO3 and PAS staining). Mannoproteins and total proteins were quantified by HPLC and by the Bradford method respectively. Finally, the foamability of the wines was measured to estimate the effect of Botrytis proteases on wine foam-active macromolecules. Results show a strong decrease of wine foamability when synthetic musts are inoculated with only 1% (v/v) of Botrytis culture. The MW distribution, the total protein and glycoprotein contents prove a strong degradation of the higher MW molecules released by *Saccharomyces* during fermentation. Mn, Mw and Hw decrease whilst Mw/Mn, the 1-500.103 g/mol fraction and MPs content (including high, medium and low MW fractions) increase. Nevertheless, no effect of cell wall mutation was observed. This study clearly shows that Botrytis is not only capable to degrade the grape proteins but also the yeast ones, with the deleterious effects on the sparkling wine foaming properties.

Application of Atmospheric Pressure Cold Plasma to wine: a preliminary study.

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The increasing consumer´s demand for free-additive wine has led the industry to consider the development and application of new technologies in their production processes. In this context, the application of atmospheric pressure cold plasma (APCP) could be an interesting tool. For this purpose, the optimization of APCP is required taking into account the inactivation of the microorganisms potentially harmful to the quality of the wine and the organoleptic impact of this technology.

In this study, APCP generated with Argon was applied for 5 and 10 min to an industrial Tempranillo wine, elaborated in the ICVV winery. The treatments were applied by triplicate in batch and in continuous with two different flows (1.2 and 2.4 l/min). After APCP treatments, the microbial population of yeasts, lactic acid bacteria and acetic acid bacteria were measured and compared to a non-treated sample of the wine. Similarly, the variation of the chromatic properties was established by measuring the indexes of Folin-Ciocalteu, polymerization and color.

Results showed that microbial populations were not significantly reduced with the applied APCP treatments. Nevertheless, some important variations in color properties were observed. A case in point was the APCP application in batch that increased significantly every color indexes with 5 and 10 min of treatment. Moreover, the APCP applied with the quickest flow produced a significant increase of the color index.

Taking into consideration those preliminary results of APCP with argon, it could be concluded that the microbial inactivation must be improved for future works. Moreover, the APCP applied in batch and with a continuous flow resulted effective, at least for some color features of wine. Overall, color properties of wines could be enhanced with this technology.

Use of membrane contactor technique to manage gas (oxygen, sulfur compounds and CO_2) in red wines: effect on sensory characteristics, acetaldehyde and phenolic compounds.

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Membrane contactors were introduced in enology for partial alcohol reduction in wine (Diban et al. 2008). Recently Schonenberge and colleagues (2014) showed that they were even effective in controlling CO_2 and O_2 concentrations in wine but no information on the effect on sensory active compounds of wine was reported. In this study Aglianico red wines were treated with a membrane contactor apparatus to: a) obtain wines with increasing levels of dissolved oxygen (0.2 mg/L, 0.5 mg/l and 1 mg/L) (O_2Ws); b) diminish and/or eliminate the reduction off-flavour in a naturraly contaminated wine (rWs); c) diminish the level of dissolved CO_2 from 3 g/L to the values of 2, 1 and 0.64 g/L in a ready for bottling wine (CO2Ws). The wines were analysed before and after the treatments. Colour indexes and phenolics were analysed by spectrophotometric methods and HPLC. Free and bound acetaldehyde were determined by HPLC after derivatization with DNPH (2,4-dinitrophenylhydrazine). Wine astringency was evaluated by sensory analysis and an astringency index based on the ability of treated wines to precipitate salivary proteins (SPI, Saliva Precipitation Index) (Rinaldi et al., 2012). For all wines no changes in chromatic characteristics and phenolic compounds were detected just after the treatments. In rWs wines a loss of the reduction off-flavour and an higher intensity of fruity notes was detected just after the treatment. A loss of free acetaldehyde was even observed. The higher the decrease of the CO_2 content in CO_2Ws wines was, the lower the percepible astringency of treated wines. No significant differences were detected in the SPI values. These results highlight that membrane-based processes are useful for the gas management of red wines.

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Taxonomic and functional traits of gut microbiomes associated with individual polyphenol metabotypes after moderate wine intake

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Polyphenol-rich foods are in the basis of a healthy diet. In this context, a moderate consumption of red wine can contribute to human gastrointestinal health, by both a direct impact in gut microbiota and by the role of it in the production of bioactive metabolites. Previous works allowed us to define, in a set of healthy human volunteers, three different phenotypes (low, moderate and high metabolizers) based on their capacity to metabolize polyphenols after a moderate and regular wine intake [1].

The main aim of this work was to co-relate the previously defined phenolic-related metabotypes, with specific gut metabolome and microbiome patterns. A targeted metabolomic approach was applied, analyzing the content of phenolic compounds, short chain fatty acids (SCFA), and immune markers in the feces of the volunteers. In parallel, the gut microbiota was analyzed by 16S-amplicon sequencing. Apart from the taxonomic assessment, the functional biodiversity was analyzed by genome matching through the Tax4Fun routine for the estimation of KEGG-based functional predictions. Multivariate analyses were applied in order to find key species/functions associated with a certain metabotype or cluster of metabolites.

The presence/abundance of some bacterial species (mainly, *Phascolarctobacterium sp., Prevotella copri, Gordonibacter pamelaeae* or *Lactobacillus reuteri*) could be co-related with a specific metabotype or with the total concentration of phenolic metabolites. Thus, they can be considered as candidate metabotype-biomarker species. Finally, there is a notable co-relation of certain groups of microbial-derived KEGG-pathways (mainly pertaining to families of glycosyltransferases, acyltransferases, and sugar permeases) with the level of specific phenolic metabolites (3-hydroxyphenylacetic acid; protocatechuic acid; 4-methylcatechol) and immune markers (Tumor Necrosis Factor; Interleukin-12(p70)), among others.

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Evaluation of the use of multiflora bee pollen as an activator of the biological aging in Sherry wines.

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Sherry wine production involves yeasts activity, commonly referred as "flor velum yeasts" that belong mainly to the species *Saccharomyces cerevisiae*. Yeast multiplication phase is one of the key moments in the "flor velum" formation. During the first stages of the biological aging, yeasts cells are found submerged in the wine where the cell walls proteins are synthesized and increase their hydrophobicity, stimulating cellular aggregates formation. These aggregates have a lower density than wine so they start to float and cover the surface forming a "biofilm" (Martínez et al., 1997).

Multiflora bee pollen addition decreases the latency times and increases cellular multiplication in white grape musts (Amores-Arrocha, Roldán, Jiménez-Cantizano, Caro, & Palacios, 2018a) and mead (Roldán et al., 2011) during alcoholic fermentation. It has been shown that bee pollen enriches yeast assimilable nitrogen (YAN) content (Amores-Arrocha et al., 2018a) in white grape musts and increase total volatile compounds production during the alcoholic fermentation (Amores-Arrocha, Roldán, Jiménez-Cantizano, Caro, & Palacios, 2018b). For these reasons, in this research work, the "flor velum yeasts" growth kinetics and yeast assimilable nitrogen in a Sherry wine supplemented with different doses of bee pollen (0-20 g/L) have been studied, to evaluate the multiflora bee pollen effect during the biological aging.

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Influence of replacing a proportion of the grape juice of very ripe red grapes by acidified water or grape juice previously treated with cationic exchange as strategy for reducing ethanol content and pH

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Grape pulp usually ripens faster than skins and seeds. Under these circumstances, winemakers can choose between two options. On the one hand, they can harvest grapes with an adequate sugar content and pH but inadequate skin and seed maturation, which will probably result in poorly-colored, bitter, astringent and herbaceous wines. Or on the other, they can wait for complete phenolic maturity and assume that their wines will have the drawback of having very high pH and alcohol content. Moreover, climate change is exacerbating this problem in recent years. Face to this problem wine industry is interested in procedures to mitigate the problem of wines with too high ethanol content and pH.

The aim of this research was to study the influence of replacing a proportion of the grape juice of very ripe red grapes by acidified water or white grape juice of low potential ethanol content previously treated with cationic exchange as strategies for reducing ethanol content and pH.

The obtained results show that both strategies enable to obtain wines with lower ethanol content and pH. However, the substitution of a proportion of the grape juice with acidified water implies a slight but significant decrease in the concentration of phenolic compounds and polysaccharides whereas when cationic exchange white must was used no significant differences were found.

It can be concluded that both techniques can be useful for simultaneous reduction of wine ethanol content and pH. However, the use acidified water is not legal in the European Union and implies a decrease in the content of some of the quality key compounds. In contrast, the use of cationic exchange white must has not these drawbacks and allows obtaining better balanced red wines.

Measurement of the oxygen transmission rate of different wine bottle closures and storage position effects.

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Oxygen is an essential component in determining the ageing potential of wine in the bottle, and the entry of oxygen into the bottle depends largely on the type of closure and the sealing efficiency of the closure. This work presents the results of the oxygen transfer rate (OTR) through some different commercial cork stoppers for wine bottles, whether natural or technical. Oxygen entry kinetics have been evaluated after bottling and also as oxygen entry kinetics during the subsequent 6 months of horizontal storage. The impact of the storage position was also evaluated. The measurement of the OTR of each closure has been made using the patented measuring system (del Alamo-Sanza M. and Nevares I., 2012) with a special adaptation for closures. The use of a standardized bottle adapter allows us to eliminate the interference of the closure-bottle neck union effect, evaluating only the OTR through the stopper. This system allows the OTR of each closure to be evaluated in a real-life situation, both with vertical and horizontal bottle position. The results indicate, in some cases, the high variability of the stopper´s OTR from the same batch and in all the cases showed the dramatic decrease in the OTR when bottle is stored horizontally.

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Evaluation of the Oxygen Transfer Rate in *Quercus Humboldtii* Oak

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Recent studies have shown the potential of *Q. humboldtii* (Colombian) as an alternative species for cooperage (Martínez-Gil et al., 2018). Since, the oxygen plays an important role in wine aging and it is known that the wood is a primary route of oxygen entry within a barrel and the species of oak is an important factor in the permeability of oxygen (Del Álamo et al., 2017), it is important to study the permeability of the news species. The aim of this study is to evaluate the OTRs of *Quercus humboldtii* untoasted and toasted. For this, the wood seasoning in open air (the usual way to seasoned the wood used for enology) and dried in oven (usual way of dry this species in cooperage) were analysed before and after toasting. The woods were evaluation under conditions functionally similar to those of a barrel oak stave, taking into account the variation in the oxygen diffusion coefficient that occurs in wood during aging. The results show the permeability of this new specie wood to oxygen, being able to know its behaviour with respect to traditional.

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Barrels with different oxygen transfer rate: a new tool for controlling the wine aging quality

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The oak barrel transfers to the wine small quantities of oxygen essential for the correct development of the wine aging process. This oxygen transfer rate (OTR) is especially dependent on the variability of the anatomy of the oak heartwood. This variability not only exists between two trees, but can also occur within a single piece of wood (stave). UVaMOX is working on the development of a non-destructive measuring system for the classification of the wood that reaches the cooper based on its OTR. This tool will allow the cooper to offer barrels with different oxygenation potential of the wines to be aged. This work presents the first results obtained from the evaluation on the properties of 16 French oak barrels made with different oxygenation potential. To do this, it was necessary to classify 3000 staves in order to choose the groups of staves with which to manufacture the barrels with different oxygenation potential in cooperage. Once manufactured, the OTR of each of them has been evaluated in order to corroborate the capacity of the system developed. The results confirm the system's ability to classify staves according to their OTR.

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Effects of pulsed electric fields treatments on the extraction of terpenoids during vinification of wine of Syrah grape.

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The grapes grown in a warm climate can provide wines with little color and a certain loss of aromas. The effect of the application of pulsed electric fields (PEF) can improve the maceration process during the vinification of red wine.

In this work, two alternatives are compared to carry out this technique during the fermentation of must of Syrah grape. In the first test was applied 60 minutes of ultrasound/day while the second test was applied double time with a total of 120 min/day.

The results showed that the wines put through to PEF had concentrations of volatile compounds greater than the control. These data coincide with the tasting exercises, where the judges highlighted the red fruit notes of the wines resulting from applied PEF in must.

On the other hand, there are differences between the two wines resulting from PEF according to the application time, being more effective the use of 60 min of ultrasound / day than applying 120min / day.

The conclusion of this research is that the application of PEF in must favors the extraction of volatile compounds in red wine and that depending on the duration of the application the effect will be different because excessive application can lead to degrading few volatile compounds.

Keywords: ultrasound, volatile compounds, Syrah, red wine, grape, PEF.

Ozone–induced changes in the skin flavanol composition of red winegrapes during dehydration

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During postharvest dehydration, the secondary metabolism of berries changes as response to osmotic and oxidative stress [1]. In withered berry skins, monomeric and oligomeric flavanols decrease in contents when dehydration progresses and therefore the flavanol biosynthetic pathway may be not induced. In addition, grape dehydration leads to an increased mean degree of polymerization (mDP). Nevertheless, varietal effects have leaded to controversial results [1]. On the other hand, ozone is a strong oxidant increasingly used in wine industry for sanitization purposes whose application on grapes can induce the synthesis of antioxidant compounds as chemical response against tissues oxidative damage [2]. In this study, the effect of the continuous treatment with 30 µL/L of gaseous ozone during grape dehydration until 10% and 20% weight loss on the flavanol profile was assessed. Both phloroglucinolysis and size exclusion chromatography were used to better evaluate the changes in the flavanol composition of two red winegrape varieties (Barbera and Nebbiolo) with different phenolic profile and contents. Barbera berry skins, characterized by lower proanthocyanidin contents, lower mDP and higher galloylation percentage than Nebbiolo, showed increased quantities of proanthocyanidins with different molecular mass in ozone-withered grapes, particularly those of low molecular mass. Instead, on Nebbiolo, ozone induced significant changes in mDP since a decrease in the quantity of the high molecular mass fractions was observed only at 10% weight loss in favor of those smallest, whereas slightly lower quantities of proanthocyanidins were found in ozone-treated grapes at 20% weight loss independently on the molecular mass. Therefore, the combined use of postharvest dehydration and long-term ozone treatment can be a strategy to induce controlled compositional changes of skin flavanols for improving wine sensory traits.

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Changes in volatile compounds of aromatic white winegrapes as response to postharvest short–term ozone treatments

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Postharvest fruits are metabolically active and can experience degradation and/or biosynthesis reactions. In fact, they react to biotic and abiotic stresses through chemical defense responses, which can be modulated to increase the phytochemical content [1]. Particularly, ozone has powerful oxidizing properties that can be exploited to its use as sanitizing agent in wine industry, but also to stimulate the accumulation of volatile compounds during grape postharvest withering under long-term ozone treatments [2]. In this study, the impact of postharvest short-term ozone treatments of Moscato bianco winegrapes (*Vitis vinifera* L.) was evaluated for the first time on volatile compounds using two different gaseous ozone concentrations (30 and 60 µL/L in air) and exposure times (24 and 48 h). Ozone effects on free and glycosylated volatile compounds were studied just after treatment (fresh grapes) and after partial dehydration up to 20% weight loss (withered grapes). The results obtained showed a significant decrease in total contents of free volatile compounds in fresh grapes, mainly terpenes. Instead, the higher oxidative stress (stronger and longer ozone treatment) may have induced the synthesis of free linalool in fresh grapes, which is one of the major aromatic markers of Moscato bianco grapes, and reduced its losses by water stress in withered grapes remaining total terpenes relatively stable. Glycosylated total volatiles and terpenes were less sensitive to ozone but a slight increase was observed in fresh grapes using the high ozone dose. Therefore, the use of short-term ozone treatments for sanitizing aromatic winegrape varieties did not change sharply the aromatic profile of fresh grapes and even improved it in withered grapes.

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Effect of High Hydrostatic Pressure (HHP) treatment on the population of *Brettanomyces anomalus*, the phenolic composition and the chromatic characteristics of a red wine in contact with oak chips

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In recent years, high hydrostatic pressure (HHP) technology has been applied to wine for microbiological stabilization purposes in different food matrices. The development of *Brettanomyces anomalus* causes the production of unpleasant off-flavour, usually named ''Brett character'' in wine. In order to reduce the levels of this yeast, HHP was applied to allow the production of wines with low levels of this microorganism. On the other hand, young red wines with a certain woody taste are increasingly appreciated and demanded by the consumer. This effect could be reproduced by the incorporation of chips during the pressurization process. The aim of this study was to analyze the effect of the application of HHP on the microbiological stability of *Brettanomyces anomalu*s and the phenolic and chromatic characteristics of a multivarietal young red wine when the treatment is applied in the presence of chips.

In a young red wine inoculated with a population of *Brettanomyces anomalus*, toasted oak chips (2.5 g/L) were added. Then, the wine was subjected to HHP treatments of 300 MPa (300CW) and 600 MPa (600CW) for 10 minutes. A semi-industrial equipment was used, Hiperbaric Wave 6000/55. After the treatment, the effect of the HHP on the general parameters, phenolic composition (total phenols, anthocyanins and flavonols), parameters related to the colour composition (copigmented anthocyanins, flavonol co-factors and polymeric pigments) and chromatic parameters (L*, a,* b*, C*, h* and saturation) were evaluated in the untreated wine (UW) and in those subjected to the HHP technique. Respect to T, the both HPP treatments did not modify the values of the general parameters (alcohol, pH, total and volatile acidity), and both caused increases of the values of flavonol cofactors, polymeric pigments and decreased the chromaticity (C*) values of wine. The HHP treatment of 600 MPa was more effective to inactivate Brettanomyces anomalus yeasts than the treatment of 300 MPa and caused the greatest differences in chromatic characteristics.

PTV and its role in the dissemination of knowledge about GTDs' and Flavescence dorée in the framework of WINETWORK project.

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The Wine Technology Platform (PTV) has collaborated in the WINETWORK (http://www.winetwork.eu/), which was an European collaborative project for the exchange and transfer of innovative knowledge between European wine-growing regions. For 3 years, 11 partners of 7 European countries have exchanged on their knowledge on two important diseases in the vineyard: grapevine trunk diseases and Flavescence dorée. Both diseases have a real economic impact in the European vitivinicultural sector. As many winegrowers are testing innovative and sustainable approaches to fighting against these diseases, it is very beneficial to capture these ideas and share them between European countries. The project approach is based on interactions between a network of 10 facilitator agents, 10 regional working groups and two scientific working groups. This participatory approach has allowed transferring results from science and practical knowledge to materials adapted to end-users. This network promoted interactions between scientists and practitioners to gather and share experiences and knowledge of different actors from the main wine producing European regions. The project was also identified the most important topics to be addressed after the end of the project, offering important replication opportunities and sustainability of the created network.

Main objectives were successfully covered by the project and the PTV:

1. Technical Working Groups (PTV, La Rioja). Technical workshops and training programs for winegrowers were carried out in La Rioja by the PTV about best cultural practices to prevent and fight against both diseases. Communication of the main findings was done during the whole project life.

2. Knowledge reservoir: participative toll which tries to collect all worldwide existing knowledge on both topics developed by research or derived from practical experience. Video seminars, Technical papers or datasheets, presentations, training documents, scientific results, images, videos, etc. can be easily uploaded by scientists, advisors or vine growers for sharing them between the community.

Replacing cold stabilisation in wineries: the use of carboxymethyl cellulose, poliaspartate and ion exchange resins

Gomez Plaza Encarna; Martinez Perez Pilar; Durant Valerie; Bautista Ortin Ana Belen

Potassium hydrogen tartrate (KHT) and calcium tartrate (CaT) are tartrate salts naturally present in wine, becoming more insoluble in wine due to presence of ethanol and during subsequent storage of bottled product at low temperatures. The tartrate crystals are not a health threatening issue, but they might be a problem for consumer acceptance and a legal issue in some countries. Therefore, treatment of wines prior bottling to prevent tartrate precipitation is an important and common step during wine production. Cold stabilization is the most common used tartrate stabilization treatment. Although cold stabilization has proven effective, it presents some significant disadvantages, such as the time needed, the high cost and environmental issues.

These are the reasons why other products are being introduce in the wineries. Some techniques are considered “subtractive” and involve reducing the concentration of tartaric acid and/or potassium in the wines. Other are “additive” techniques and make use of protective colloids or crystallization inhibitors that can be added to the wine. In this way, among additive processes, carboxymethyl cellulose and the newly approved poliaspartate inhibit the tartaric acid crystallization whereas the subtractive methods such as the ion exchange resins eliminates K and Ca in the wine. These methodologies can be really effective although also some problems can occur such as colour instability in red wines or sensory defaults.

In this study, white, rose and red wines were treated with CMC, polyaspartate and ion exchange resins and they were evaluated after the stabilization treatment. Enological and chromatic characteristics have been evaluated, the results indicating that sensory and chromatic characteristics were maintained in the wines (compared with a control non-treated wine), differences not being detected in an triangular sensory test. Pros and cons of each technique will be further evaluated.

Making wine healthier with UV-C treatment during maceration

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Wine is often consumed nowadays, and its benefits to health are undeniable. In recent years, conscious consumers want to take advantage of their antioxidative effects as well as organoleptic properties of wine. Especially red wines with high value of phenolic compounds than white wines have positive effect on our health. Phenolic compounds in red wines are responsible for color, bitterness, astringency, and aging behavior of wine. The studies confirmed that antioxidant phenolic compounds demonstrate cardioprotective, vasorelaxative, anti-inflammatory, reactive oxygen scavenging, and anticancer activities. Wine-making techniques have an influence on phenolic content of completely fermented red wines, which determines wine quality. This research was conducted to increase the content of phenolic compounds by UV-C irradiation applied during the maceration of wine obtained from grape varieties containing low phenolic compounds. Kalecik Karası (*Vitis vinifera* L.) winegrape cv., that is most widely used for winemaking in Turkey is used in this research. Total phenolic and anthocyanin contents and trolox equivalent antioxidant capacity of wines were measured spectrophotometrically and phenolic contents (+)-catechin, (–)-epicatechin, rutin, quercetin, *trans*-resveratrol, and *cis*-resveratrol were measured by High Pressure Liquid Chromatography with Diode Array Detection (HPLC-DAD). According to the results of the study, all parameters were obtained with the highest UV-C applied wine.

Key words: Red wine, Kalecik Karası, phenolic compounds, UV-C application, health.

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